OCCURRENCE AND ANALYSIS OF CYTOMORPHOLOGICAL FEATURES OF PAPILLARY CARCINOMA THYROID IN ALL OTHER THYROID SWELLINGS

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the requirements for the award of the degree of

M.D in PATHOLOGY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCE & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

CERTIFICATE

This is to certify that the dissertation work entitled "**Occurrence And Analysis Of Cytomorphological Features Of Papillary Carcinoma Thyroid In All Other Thyroid Swellings"** submitted by **Dr Jacinth Babu,** is a work done by him during the period of study in this department from 30/05/2012 to 29/05/2015. This work was done under the guidance of **Dr. Chetna Sharma,** Associate Professor, Department of Pathology, PSG IMS&R.

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CERTIFICATE

This is to certify that the thesis entitled "**Occurrence And Analysis Of Cytomorphological Features Of Papillary Carcinoma Thyroid In All Other Thyroid Swellings"** submitted by

Dr Jacinth Babu to The Tamilnadu Dr MGR Medical University, Chennai, for the award of the degree of **Doctor of Medicine in Pathology,** is a bonafide record of research work carried out by him under the guidance of **Dr. Chetna Sharma**. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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Date: 24/09/2014

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November 22, 2012

To
Dr Jacinth Babu
I year Post Graduate
Department of Pathology
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Coimbatore

Ref.: Your study entitled 'Occurrence and analysis of cytomorphological features of papillary carcinoma of thyroid in all other thyroid swellings'

Ref.2: Our letter dated 19.09.2012
Documents submitted by you on 28.09.2012 and 19.11.2012

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 18th September, 2012 in its expedited review meeting held at College Council Room, PSG IMS&R, between 3.00 pm and 4.30 pm, and discussed your application to conduct the study entitled:

"Occurrence and analysis of cytomorphological features of papillary carcinoma of thyroid in all other thyroid swellings"

The following documents were received for review:

1. Duly filled application form
2. Procedure for anonymization
3. Confidentiality Statement
4. Data Collection Tool
5. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting held on 18.09.2012, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist	Female	Yes	Yes

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		Member - Secretary			
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr Y S Sivan	Ph D	Member - Social Scientist	Male	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the full board review meeting scheduled on 30.11.2012.

Yours truly,

22-11-12

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

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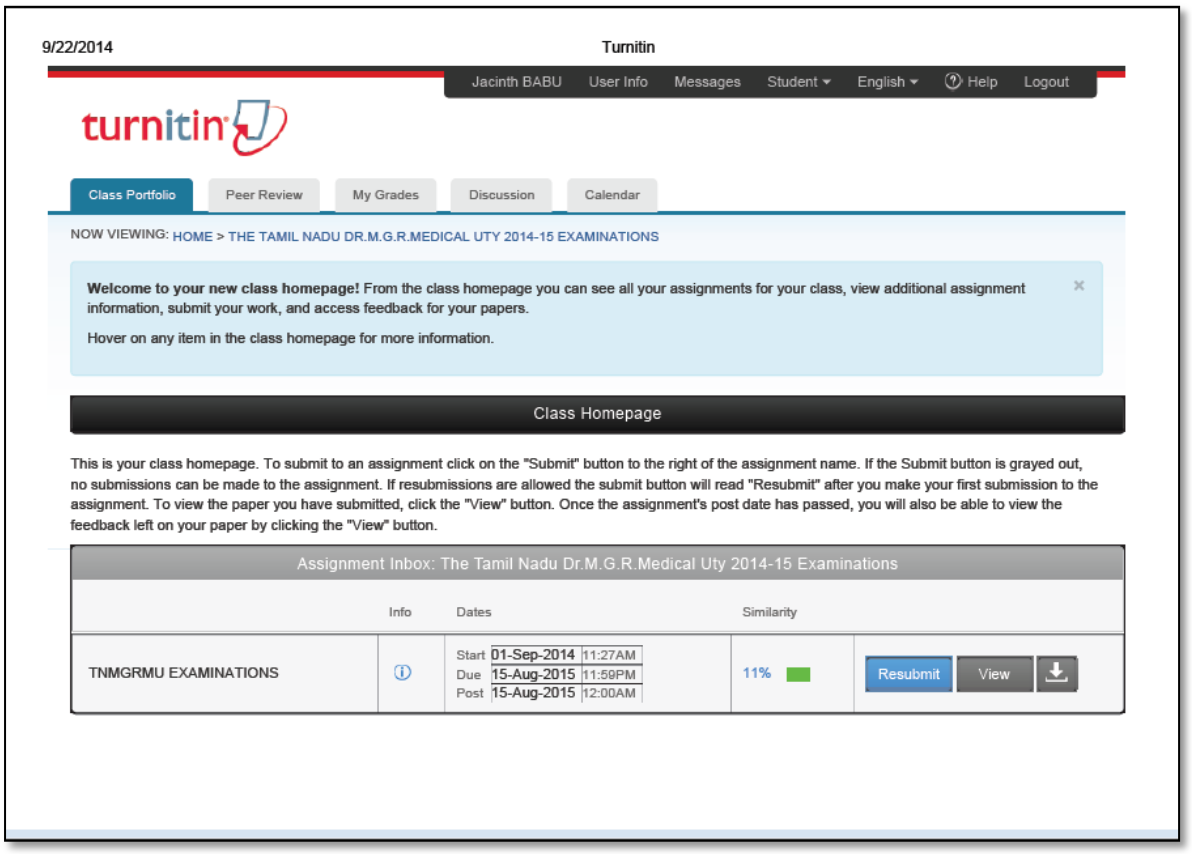
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ACKNOWLEDGEMENT

"For this, O LORD, I will praise you among the nations;

I will sing praises to Your name." Psalm 18:49

I would like to thank and praise my ***Heavenly Father***, for enabling me to complete this thesis which seemed like a mountain and leading me preciously every step of the way.

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"A family is a circle of strength, founded on faith, joined in love and kept by God". I am blessed with a wonderful family which includes a big circle of; my ***parents, in-laws, siblings and relatives*** who have been my constant support, encouragement through thick and thin. Words cannot express my gratitude. Your prayers and blessings have brought me till here and I am deeply grateful for everything.

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OCCURRENCE AND ANALYSIS OF CYTOMORPHOLOGICAL FEATURES OF PAPILLARY CARCINOMA THYROID IN ALL OTHER THYROID SWELLINGS

Abstract:

Papillary Carcinoma is the most malignant tumor among all thyroid cancers. In contrast to its high incidence, death due to papillary carcinoma is rare as they respond well to treatment. Fine Needle Aspiration is the first line diagnostic test for evaluation of any thyroid swelling and papillary carcinoma thyroid can be effectively diagnosed by it.

In our study, five cytomorphological features namely: Nuclear grooves, Pseudoinclusions, Papillary fragments, Metaplastic cytoplasm and Three Dimensional fragments were selected and analysed. A quantitative and semi-quantitative assessment was done for the nuclear grooves and intra-nuclear inclusions respectively. Subsequently, Sensitivity and Specificity of each of the above mentioned features was statistically derived.

We concluded that presence of ≥ 20% Nuclear grooves with frequent Pseudoinclusions is virtually diagnostic of Papillary carcinoma. The cases which fall in the category of 10-19 % nuclear grooves should be considered as Suspicious for malignancy and in cases with < 10% Nuclear grooves, the possibility of Papillary carcinoma can be ruled out.

Keywords: Papillary carcinoma, Nuclear grooves, Pseudoinclusions, Papillary fragments, Metaplastic cytoplasm and Three Dimensional fragments, Thyroid FNA.

INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is the firstline diagnostic test for the evaluation of swellings of the thyroid gland. It is an efficient method of selecting patients who require surgical treatment owing to its simplicity, low cost, high sensitivity and specificity. Despite these advantages, Fine Needle Aspiration Cytology can be limited by the quality of the material available for assessment.

Thyroid cancer is the most common endocrine malignancy and represents 1% of all malignancies. Papillary thyroid carcinoma (PTC) is the most common malignant tumor among all thyroid cancers, comprising an estimated 80% of thyroid cancers. In contrast to the high incidence, death from papillary carcinoma is rare, and most patients respond to surgery and targeted therapy with radioactive iodine.

The diagnostic criterion for papillary carcinoma thyroid is well established. But no single criterion is specific; as they may be found in other causes for goitre too. We therefore wish to analyse all non-papillary carcinoma thyroid cases for the presence of cytological features which are otherwise diagnostic of a papillary thyroid carcinoma.

AIM AND OBJECTIVE

- To observe the presence of cytomorphological features of papillary carcinoma in FNAC smears of all thyroid swellings that also have a tissue diagnosis.

- To analyse the diagnostic significance of these features in the diagnosis of papillary carcinoma of thyroid by using a quantitative/semi-quantitative approach.

EMBRYOLOGY:

The human thyroid initially appears as a median anlage and two lateral anlagen. Thyroglossal duct develops as a duct like invagination from the primitive pharynx at the foramen caecum which is located at the floor of the primitive pharynx. The thyroid gland which is initially spherical in shape develops at the base of the thyroglossal duct. Later when approaching the final stage, the thyroid becomes bilobed and comes to lie in front of the trachea by about 7 weeks of gestation[1].

These solid thyroid anlagen start to form cords and plates of follicular cells by about 9th week of gestation and small follicles are evident by about 10th week. Following the development of small follicles, a finely granular material begins to collect within these primitive follicles which get the morphological features of colloid by 20th week. Well developed follicles with central lumen containing colloid can be seen as early as 14th week of gestation[1].

Cytoplasm of the follicular cells and intraluminal colloid are thyroglobulin positive. Many studies show that thyroglobulin secretion starts much earlier, even before the formation of follicles and colloid, when the thyroid is still a solid mass at the base of tongue[1].

One of the factors which helps in the maintenance of functional differentiation in follicular cells is PAX8 which has been demonstrated in median thyroid anlage, thyroglossal ducts and in ultimobranchial bodies[2]. Thyroid Transcription Factor-1 (TTF1) is another factor which is also expressed in the median thyroid anlage. Ghrelin (growth hormone releasing hormone) which can be demonstrated immunohistochemically is identified in the follicular cells of foetal thyroid.

The two lateral anlagen derive from the ultimobranchial bodies and become separated from the pharynx and the parathyroid by 7^{th} to 8^{th} week. By 9^{th} week their lumen become obliterated by the proliferating cells and appear as solid masses that fuse with the median thyroid anlage and later becomes incorporated to the developing lateral lobes[3]. C cells are thought to be derived from neural crest and then move to the ultimobranchial bodies before they get incorporated into the thyroid.

The rate of development of foetal thyroid is very rapid till the 4^{th} month of intrauterine life (i.e) till the crown – rump length reaches 18mm. Immediately after birth the rate of growth parallels to that of the body. The normal adult weight of thyroid is reached by about 15 years of age[1].

GROSS ANATOMY:

The thyroid tissue is situated in the mid portion of the neck and is attached to the anterior trachea by loose connective tissue. The entire thyroid gland along with the lateral lobes covers the second, third and fourth tracheal rings[1].

A normal adult thyroid gland weighs approximately 15 to 25 grams. This can vary with individuals in relation to age, gender, hormonal status, functional status of the gland and iodine intake. In women, volume of thyroid tends to increase during the secretory phase of menstrual cycle[4].

The normal adult thyroid with two lateral lobes connected by isthmus gives the appearance of a butterfly. Approximately each lateral lobe measures 5-6 cm in length, 2-2.5 cm in width and 2 cm in depth. Thyroid has two poles, the upper extremity referred to as upper pole and the lower extremity referred to as lower pole. The upper pole is pointed and the lower counterpart is usually blunt. About 40% of the thyroid has a narrow projection upward from the isthmus which is a vestige of the thyroglossal duct, known as pyramidal lobe[1].

The colour of normal adult thyroid is red – brown[1].

The thyroid gland is seen to be invested by a thin fibrous capsule. Numerous fibrous septa arise from this capsule, which seems to penetrate the thyroid

parenchyma dividing it in to lobules. These individual lobules are known as thyromeres[1].

Thyroid gland receives its blood supply mainly from inferior thyroid artery and superior thyroid artery which originates from the thyrocervical trunk of subclavian artery and external carotid artery respectively. Another artery known as thyroidea ima artery may also be present. The venous drainage is by venous plexus which are present in the thyroid capsule draining in to the superior, medial and inferior thyroid veins which further drains in to the internal jugular and then into the brachiocephalic vein[5].

The lymphatic network of thyroid is seen to traverse the gland encircling the follicles. This network empties in to the sub capsular channels which in turn drain in to the collecting trunk. Lymphatics draining the superior portion of thyroid, drains in to internal jugular lymph nodes and those draining inferior portion of the gland, drains in to pre and paratracheal and prelaryngeal lymph nodes. There are certain type of dye injection studies which show that isthmus can drain directly in to the mediastinal nodes[6].

Thyroid secretions are mediated by two pathways; one is directly by the neural signals and the other by indirect vascular nerve signal. The indirect secretory activity is mediated by the postganglionic neural fibres which arise from the superior and midline cervical sympathetic ganglia, through their action on blood vessels[6].

MICROSCOPIC ANATOMY:

The basic unit of thyroid is follicle. These follicles are round to oval and are lined by a single layer of epithelial cells. Lumen of the follicle is filled by a viscous material called colloid. These follicles are kept apart by loose fibro connective tissue.

In a resting follicle, the colloid acquires a deeply eosinophilic staining quality and in an actively secreting gland, the colloid appears pale eosinophilic with scalloped border. This results in a vacuolated appearance in between the epithelium and the colloid, known as resorption vacuoles. Occasionally, colloid can occur as round basophilic corpuscle clusters at one end of the follicle.

The glycoprotein material which is present with in the follicles stains for Periodic acid- Schiff (PAS) and Alcian blue.

The follicles are lined by epithelial glandular cells known as follicular cells or thyrocytes. A second cellular component known as C cells is seen interspersed between the thyrocytes[1].

Follicular Cells:

Follicular cells can show three different morphologic patterns depending on the functional status of the gland. Inactive follicles are lined by flattened cells. Cuboidal cells are commonly seen and their major function is secretion of

colloid. The columnar cells are seen rarely and when present help in resorption of thyroglobulin containing colloid, liberation of hormones and their excretion in to the blood vessels. This gives the appearance of apical lipid droplets or basilar vacuoles known as vacuoles of Bensley[1].

All follicular cells are oriented basally on the basement membrane with their apices directed towards lumen of the follicle. The nucleus is round to oval and is centrally placed with eccentrically located nucleoli. The chromatin pattern is fine or clumped. The cytoplasm is slightly eosinophilic. Occasionally the cytoplasm shows a lipofuscin type of golden brown pigment.

- **Immunohistochemistry**:

Thyroglobulin: is the most specific marker for normal follicular cells and tumor cells derived from them. The antibodies used can be either monoclonal or polyclonal and the reactivity can be seen both in cytoplasm and colloid[1].

Thyroid transcription factor-1(TTF-1): is the next useful marker for demonstration of follicular epithelial cells and the tumors developing from it. This factor is seen in thyroid follicular cells and also in pneumocytes. The distribution of this factor is related to thyroglobulin and thyroperoxidase[7].

Some of the other immunohistochemical markers which can show variable expression with the thyroid follicles are keratins, vimentin, epithelial membrane antigen, triiodothyronine and thyroxine, estrogen and progesterone

receptors, S-100 protein, epidermal growth factor receptor, thyroid peroxidase and sodium iodide symporter[1].

- **Physiology:**

The important function of thyroid gland is production of thyroid hormones, thyroxin (T4) and triiodothyronine (T3). These thyroid hormones are responsible mainly for the growth of the body. They are also helpful for normal maturation of central and peripheral nervous system, to regulate protein metabolism and to increase oxygen consumption[1].

C cells (Parafollicular cells):

They represent a minor component of the thyroid gland, comprising less than 0.1% of the cellular component. C cells have neuroendocrine function by producing a peptide hormone Calcitonin (CT).

The identification of C cells in hematoxylin and eosin stained section is difficult. They are polygonal with granular eosinophilic cytoplasm. The nucleus is round to oval with a central nucleolus.

They are located singly or in groups within the thyroid follicle, most of which are found at the periphery of the follicular wall without any contact with the follicular lumen[1].

Number of C cells varies with development of the gland. It is said that C cells are more numerous in the early age. According to some studies neonates and children show up to 100 C cells / LPFwhere as in adults only a maximum of 10 cells were counted in 10 LPF[8].

- **Histochemistry and Immunohistochemistry:**

Histochemically, C cells are characterised by Argyrophilia, Lead hematoxilin, Toluidine blue

Immunohistichemically, C cells are found to be reactive to Calcitonin, Calcitonin-gene related peptide, Katacalcin, Somatostatin, substance P, serotonin and other biologically active amines, Gastrin releasing peptide, pure – endocrine markers, cytokeratins and carcinoembryonic antigen[1].

- **Physiology:**

The main function of calcitonin is regulation of calcium level in the plasma by a feedback mechanism inhibiting the osteoclastic activity. Hence, its major physiological role is to protect the skeleton during stress as in pregnancy and lactation[9]. Calcitonin also increases the production of vitamin D by acting on the kidneys.

Stroma:

Lymphocytes: can be seen scattered or as a collection in the interstitium. In addition to lymphocytes, plasma cells can be seen admixed occasionally.

Fibrous septa: are seen to separate the thyroid lobules. Usually they are thin, but can also show dense, acellular collagen fibres dividing the thyroid tissue into smaller nodules.

Mature adipose tissue: can be seen in a normal follicle as a result of adipose metaplasia. Sometimes adipose tissue can be seen in close proximity to the thyroid capsule resulting from the close relationship of fat and thyroid tissue during the foetal life.

Cartilage and muscle: are other tissues that can be found within the capsule of the adult thyroid as a result of their close proximity to the thyroid gland during development.

In old age, dystrophic calcification can be seen in close relation to the blood vessels. This type of calcification can be easily distinguished from psammoma bodies due to their lack of laminations and irregularity of their contour[1].

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC):

The practice of Fine Needle Aspiration Cytology has prevailed among the Scandinavian countries for the past six decades. The initial fear of Fine Needle Aspiration procedure among the pathologists has gradually diminished. This technique of Fine Needle Aspiration came to use in United States and United Kingdom during seventies. Now it is practised worldwide[10].

Among the thyroid lesions, solitary thyroid nodule poses a clinical problem as it has the tendency to turn malignant when compared to that of multinodulargoitre[10]. Clinical examination cannot be relied to distinguish solitary and multinodular goitre as most of these cases which appear solitary actually represents a dominant nodule in a multinodular goitre.

Some of the non- invasive screening techniques used to evaluate the clinically solitary nodules are ultrasonography (USG), thyroid scinti-scanning, thyroid function test, thyroid antibodies and thyroid hormone suppression test[10].

Ultrasonography helps to determine, if the thyroid nodule is solitary and then to further categorize them into solid, cystic or mixed nodules. Amongst these categories, solid nodules have higher tendency to turn malignant[11].

However, ultrasound appearance may be misleading sometimes as solid lesion can be mistaken for cyst and rarely cyst being mistaken for solid lesion.

Hence ultrasonography is not an appropriate primary investigative modality for thyroid nodules.

Thyroid scinti-scanning helps in identifying cold nodule. 75-90% of solitary thyroid nodules are cold and most of them are benign[11] but this cannot definitely differentiate between benign and malignant nodules.

Thyroid function test and thyroid antibodies have little or no role in the diagnosis of solitary thyroid nodule[10].

Thyroid hormone suppression has been tried using the rationale that involution is indicative of benign lesion, however anything less than complete involution does not rule out the possibility of malignancy. Conversely, absence of involution is an unreliable indicator of malignancy[12].

To overcome the above said difficulties in defining a solitary nodule and further categorising them as benign or malignant, we need a better investigative modality. After the advent of Fine Needle Aspiration Cytology, this has become the better mode of investigation for distinguishing benign and solitary nodules[10].

FNA CYTOLOGY:

Before the advent of FNA cytology, most of the thyroid nodules were surgically excised and the incidence of malignancy in these excised thyroid nodules ranged between 15 and 50%[13].

The role of FNA cytology is to select cases that necessitate surgery and to provide a pre-operative morphological diagnosis that can prevent unnecessary surgery and help to plan appropriate surgery and treatment protocols. Since the advent of FNA cytology, the number of patients undergoing thyroid surgeries all over the world has dropped to almost 50%[10].

Hence FNA cytology has unquestionably become the single most screening method for thyroid nodules, as it is cost- effective and also has the advantage of accurate morphological typing of thyroid tumors.

In a research article published by Manoj Gupta and team, a correlation study between Fine needle aspiration cytology and Histopathology in the diagnosis of solitary thyroid nodule was done and they concluded that a FNAC diagnosis of malignancy was highly indicative of surgery but patients labelled benign by FNAC findings should be followed up regularly. If there was any suspicion of malignancy even in the presence of benign FNA diagnosis, surgery should be mandated[14].

DIAGNOSTIC PITFALLS:

- Confusion occurs specially in cystic nodules. Hence a systematic approach is necessary while handling a case of cystic nodule or any thyroid lesion co- existing with cystic lesion
- There is also a possibility of false positive cases in relation to cystic thyroid lesions[15].
- False positivity is also common among patients with Hurthle cell component[16].

FINE NEEDLE CAPILLARY (FNC) SAMPLING:

This is an alternative to FNA cytology where sampling is done without aspiration, the technique being called as non aspiration fine needle technique.

PROCEDURE FOR THYROID FNA:

The needle used for FNAC is 22-23 gauge disposable needle which is attached to a 20ml disposable plastic syringe. This syringe is mounted on a syringe holder for a single hand grip.

To start with, the patient is asked to lie in the supine position with the neck in hyperextended state and this can be facilitated with the help of a pillow under

the neck. During the procedure, the patient is asked to refrain from swallowing.

The nodule is palpated and the skin over the nodule is cleaned with alcohol and the needle is gently inserted into the nodule. As soon as the needle is inserted, the plunger is retracted to create a negative pressure in the syringe. The needle is then moved back and forth and also side to side gently within the lesion. The negative pressure is maintained as this helps to loosen the cells and enable easy suction of cells into the needle.

In a perfect FNA technique, the cells are just visible in the hub of the needle but do not enter the syringe barrel[10]. The needle with syringe is then withdrawn from the thyroid.

The needle is then detached quickly and the syringe holder is retracted to fill the barrel of the syringe with air. Then the needle is reattached and the contents are pushed on to the glass slide by resting the tip of the needle on the glass slide.

A glass slide or a cover slip can be used to spread the aspirated material on the glass slide[10].

An ideal smear should be approximately oval in shape and should occupy an area of 70-80% of the slide[10].

The cytopathologist can assess the adequacy of the material obtained, by quickly staining one of the smears by the bed side itself. After assessing the adequacy, a few more passes can be made if the cellularity is inadequate.

In cystic nodules some of the following protocols help to improve the yield of cellularity[17].

- Two or more pricks are made from different places in the nodule and the maximum possible amount of fluid aspirated.
- The fluid is then centrifuged. The supernatant fluid is discarded leaving behind a small amount to re-suspend the deposits. The re-suspended material is then used for preparing cytospin slides.
- The last few drops in the needle can be used for smearing but care should be taken not to make a very thin smear out of it.
- If there is a residual palpable mass after aspiration, re-aspiration should be performed.

According to certain studies, if the deposits are thick after centrifugation, a cell block can be prepared to minimise the risk of missing cystic papillary carcinoma.

Some of the other techniques performed in cytology are Cytospin, ThinPrep, Vassilakos method and FNA-21 method.

FIXATION AND STAINING:

The smears thus obtained by the above method are air dried, fixed in methanol and stained with Wright Giemsa stain or May Grunwaldstain[10]. Some cytopathologists in different parts of the world prefer Papanicolaou (Pap) stain and Haematoxilin and Eosin (H & E) staining methods. In our institution we use Wright Giemsa, Papanicolaou stain and H & E stain routinely for all the cases.

The main advantage of Giemsa stain is that, the step of air- drying helps in preventing cell drop out. Papanicolaou stain exhibit nuclear characteristics such as chromatin, nuclei and nucleoli better in wet preparation. The main disadvantage of Papanicolaou stain is that the ethanol fixation step causes cell shrinkage and also some degree of cell drop out.

If the Diff-Quik stained smear suggests high cellularity and a neoplasm then more material should be collected in 95% ethanol to prepare cell-block. A 22 gauge needle is used for aspiration to prepare a cell-block. The fluid thus collected is centrifuged and the cell block is prepared from the deposits. Sections cut from the paraffin block, which is prepared from the cell-block can be used for routine H & E stain, Papanicolaou stain, special stain or immune stain[10].

EVALUATION OF THE STAINED SMEAR:

The smear is first scanned with 4x objective looking at the background (bloody, colloid stained or mixture of both), then the cellularity (highly cellular, moderately cellular, mildly cellular or scant) and lastly the cell pattern (i.e.) arrangement in monolayered sheets, three dimensional clusters, papillary pattern or acinar pattern. With 4X, the observer can also select the field for examining at higher magnification.

After selecting the field of interest at 4X magnification, the observer moves on to 10X for better appreciation of cell pattern and then to 40X for better cellular details[10].

Appropriate clinical details and laboratory data are necessary while interpreting cytological smears for accurate reporting.

SPECIMEN ADEQUACY:

Cytologically, benign appearing lesions can be called adequate if there are atleast six clusters in minimum of two slides[18]. Some studies state that a minimum of 10 – 15 clusters of benign follicular cells in atleast six slides[19] are required to call it adequate. In our institution we follow the first criteria for specimen adequacy.

However in literature, there are no clear cut criteria for specimen adequacy in neoplastic lesion. If there are a very few clusters, but with the characteristic features of a particular tumor, then the number of clusters do not matter to give a diagnosis of that particular tumor.

If there is possibility of giving a clinically relevant cytologic report, thenthe adequacy relies on a cytologically diagnostic sample and the above criteria does not hold good in such cases. For example, in certain cases like diffuse nodular goitre and non-neoplastic cystic lesion, the follicular epithelial cells can be very few or scant except for the presence of colloid or exclusive population of foamy cells (Histiocytes) in the background.

REPORTING OF SMEAR:

While reporting, a standard format containing a list of cytomorphological parameters under various headings can be used for simple and systematic reporting. According to Gita Jayaraman's book on Atlas and Text book of Thyroid Cytology, reporting format *(table 1)* for thyroid cytology can be simplified as follows;

Table 1: Reporting Format

Background	Cellularity	Pattern	Follicular cells	Foam cells	Other cells
Scant colloid	Good	Clusters	Monomorphic	Few	Spindle cells
Abundant colloid	Moderate	3-D	Pleomorphic	Many	Round cells
Blood	Mild	Papillary	Associated with colloid globi		Tumour giant cells
Lymphoid cells	Scant	Acinar	Degenerative changes	Metaplasia	Sarcomatoid cells
		Dissociated	Infiltration by lymphocytes	Foam cell	Langhanoid giant cells
			Fire flare	Hurthle cell	Foreign body giant cells
			Intranuclear inclusions/ grooves	squamous	Epithelioid granuloma
Other features (specify) :					

THE CELLS IN CYTOLOGY:

In a thyroid tissue, the functional unit is follicle. This follicle is composed of follicular epithelium with central colloid. The basic two elements of the follicle are follicular cells and colloid and are important in the diagnosis of most of the thyroid lesions.

- *Follicular cells:*

The size and shape of normal follicular cells change depending on the functional activity of the gland. Hence the gland appears low cuboidal when the gland is inactive and tall, columnar in hyperactive state[20].

Follicular cells resemble endocervical cells in having uniform cells which are cohesive and arranged in honey comb pattern[21]. This type of pattern is characteristic of non - neoplastic lesion. The nuclei do not show crowding or overlapping.

The nuclei vary in shape from round to oval. Their size varies from 6μm to 12 μm in diameter. The nucleus is almost the size of lymphocyte, and hence a bare follicular nucleus can be easily mistaken for a lymphocyte[22]. Nuclear size varies with the functional state and also with age of the patient. Nuclear moulding is not seen in a non - neoplastic thyroid. The nuclear membrane is usually smooth in benign conditions. The chromatin pattern is normally granular with uniform distribution. Nucleoli are usually inconspicuous. It is

noted that normal cell division occurs once in eight or nine years[23] and hence mitotic figures are not seen commonly.

The cytoplasm usually appears pale and delicate in the Papanicolaou stain and light blue to purple in the Ramanowsky stain. The cytoplasm is very fragile and can be stripped leaving behind naked nuclei[24]. In normal follicular cell, cytoplasmic borders are not prominent. Dense cytoplasm with evident cell borders suggests abnormality.

Ultrastructurally, the follicular cells are seen to have plenty of rough endoplasmic reticulum, well developed golgi apparatus, lysosomes and microvilli which are seen along the luminal border[25]

The follicular cells on degeneration resemble the morphology of histiocyte and appear indistinguishable from them, as the cytoplasm becomes foamy and granular on degeneration. In case of old bleeding, the cytoplasm will show hemosiderin granules[27].

Flame cell:

Flame cell got its name because of its fire-flame appearance when stained with Ramanowsky stain[26]. These cells have abundant, vacuolated cytoplasm. The vacuoles vary in size and can reach up to the size of a RBC. These vacuoles contain metachromatic material which is responsible for the flame like appearance of these cells. The above said changes are thought to be due to

dilated endoplasmic reticulum which is associated with raised protein synthesis in active follicular cells[27].

- *Hurthle Cell:*

Hurthle cells are large polygonal cells. They have abundant, granular cytoplasm. Ultrastructurally these granules represent mitochondria. Hence if the cytoplasm of a cell is abundant but fibrillary in nature rather than granular, then they cannot be qualified as Hurthle cells. The nuclei tend to be eccentrically placed and are enlarged 2 – 4 times the normal size. Frequently the nucleus exhibits binucleation or multinucleation. The chromatin pattern can be fine to coarse with occasionally large and prominent nucleoli[27].

C cells and parathyroid cells cannot be differentiated from the follicular cells in fine needle aspiration cytology or biopsy. A special technique may be required to demonstrate these cells.

- *Multinucleated giant cells:*

Multinucleated giant cell histiocytes can be seen in many of the thyroid lesions such as inflammatory conditions, hyperplastic conditions and in some neoplastic disorders. These cells have abundant vacuolated or foamy cytoplasm with multiple nucleoli sometimes ranging more than a dozen in a single cell[27].

- *Lymphocytes:*

Lymphocytes can be present along with other chronic inflammatory infiltrates in most of the thyroid conditions. Naked follicular nuclei can be confused with lymphocytes[27].

- *Ciliated cells:*

Ciliated cells are not normal constituents of thyroid gland. If present, it is usually due to contamination of the aspirate by the needle which inadvertently entered the trachea and aspirated the respiratory epithelium.

Other cells like squamous cells, spindle cells, blood vessels, skin and rarely hematopoietic elements can be seen in thyroid aspirates[27].

- *Colloid:*

Colloid is a glycoprotein that is Periodic Acid – Schiff positive and acts as sites for the storage of iodinated thyroid hormones. Grossly, they have the consistency of honey.

Microscopically, colloid may be of one of the following two forms; watery (diffuse) or dense (solid). Watery colloid appears thin, homogenous and acellular on microscopic slides. Sometimes, only the outline of colloid remains giving the appearance of spider web or chicken wire. If the colloid is lost during processing, presence of rouleaux formation would suggest the loss of colloid[27]. In order to differentiate colloid from other substances such as blood

and serum, an area away from the edges and clots should be examined[28]. Unlike watery colloid, the dense colloid can be easily identified as it appears rounded and homogenous that stains deep purple with Ramanowsky stain [27]. However, skeletal muscle can be mistaken for dense colloid.

- *Mucin:*

According to a study conducted by E Foster in 1963, mucin secretion was strongly considered to rule out the possibility of tumor origin in thyroid, but now it is regarded that mucin secretion is more commonly seen in thyroid tumors than in other non - neoplastic thyroid lesions[29].

- *Granules:*

Granules can be of two types, red granules and blue granules. Red granules (metachromatic) can either be in the cytoplasm of the neuroendocrine cells or in the cytoplasm of flame cells where they are characteristically apical in location. Blue granules are paravacuolar granules and blue bodies. Paravacuolar granules are lysosomes containing hemosiderin or lipofuscin. It is usually a non specific finding. Blue bodies are intermediate filaments seen in the cytoplasm of benign follicular cells[27].

- *Pigments / crystals:*

Hemosiderin pigments appear golden – brown while staining with Papanicolaou stain and dark blue in Ramanowsky stain. They are seen to be

associated with bleeding. Melanin like pigments which stain positive for Fontana – Masson silver stain can be seen associated with tetracycline therapy. Pigmentation can also be associated with hemochromatosis, ochronosis and haemorrhage[27].

- *Calcification:*

Calcification can be seen both in benign and malignant conditions. Calcification will be of two forms: dystrophic and psammomatous. Dystrophic calcification can be further divided in to egg shell calcification and nodular calcification[30]. Psammoma bodies are made up of calcium apatite. They appear as concentrically laminated crystalline structures. Psammoma bodies are most commonly associated with papillary carcinoma thyroid. It can also be seen in other non - malignant conditions[27].

- *Amyloid:*

Amyloid is seen as acellular, amorphous material that simulates colloid in appearance. It appears eosinophilic to cyanophilic in Papanicolaou stain and metachromatic with Ramanowsky stain.

DISEASES OF THYROID

According to Koss' Diagnostic cytology, some of the lesions of thyroid that can be diagnosed in aspiration cytology are as follows:

1. Cysts
2. Goitres
    a. Colloid Goitre
3. Thyroiditis
    a. Acute
    b. Subacute (De Quervain's)
    c. Lymphocytic (Hashimoto's disease)
    d. Riedel's Struma (fibrosing thyroiditis)
4. Tumors
    a. Follicular tumors
        i. Adenoma
        ii. Carcinoma
    b. Hurthle cell tumors
        i. Adenoma
        ii. Carcinoma
    c. Other carcinoma
        i. Papillary and its variants
        ii. Medullary

iii. Anaplastic (Large and Small cell type)

d. Malignant lymphoma
e. Rare Malignant tumors
f. Metastatic tumors

BENIGN THYROID DISEASES

Cysts:

According to Koss, cystic lesions contribute around 10% to 30% of all the thyroid nodules and among these cystic lesions almost all are pseudocysts developing either from nodular goitre or arising in adenomas.

Very rarely true cysts may be seen to derive from the remnants of thyroglossal duct[33]. It is noteworthy to state here that some malignant tumors, specifically papillary carcinomas tend to be partly cystic.

It is also suggested here that if a palpable mass remains even after aspiration of the fluid, aspiration should be repeated to rule out the possibility of tumor masked by the cyst[33].

According to Marluce Bibbo, some of the key features of cysts are;

- Dark brown, blood stained or transparent yellow fluid in varying amount

- Presence of degenerated follicular cells
- Presence of foamy macrophages
- Rarely atypical looking cells can also be seen.

According to Nassar et al. these atypical cells could be sheets of macrophages exhibiting chromatin clearing and intra-nuclear grooves[31]. But Faquin et al. demonstrated that these flat sheets are nothing but reactive follicular cells which were evenly spaced and covered atleast part of cyst wall[32].

Goitre:

Goitre literally means enlargement of the thyroid gland due to any of the reasons such as colloid goitre or inflammation or neoplastic condition[33].

- *Colloid goitre:*

Colloid goitre is induced by iodine deficiency which results in hyperplasia of the thyroid gland.

According to Marluce Bibbo, some of the key features are;

- Abundant amber coloured colloid
- Follicular cells which are scanty and forming loose follicles with smooth contour.

The colloid in most cases can be seen to be mixed with blood and gives the appearance of mosaic like crackling.

Practically, if there is an enlarged thyroid gland with colloid rich smear and normal appearing follicular cells, it is suggestive of colloid goitre[35].

Some cases of papillary carcinoma and follicular carcinoma can also show abundant colloid. It is very rare to identify C cells in a normal or hyperplastic thyroid gland, but if present they may resemble the cells of medullary carcinoma[35].

Thyroiditis:

Thyroiditis involve the inflammatory conditions of the thyroid and encompasses a wide group of diseases ranging from acute suppurative thyroiditis to chronic inflammatory processes[33].

- *Acute thyroiditis:*

Patients with acute thyroiditis clinically present with erythematous, highly tender thyroid accompanied by fever. On examination, the thyroid is diffusely enlarged. The aspirates will show follicular cells, neutrophils and macrophages. A study conducted by Guttler and Singer in 1988 found that Pneumocystis carinii in AIDS patients caused thyroiditis. Also Sodhani in 1989 reported the evidence of microfilariae in cases of acute thyrioiditis[33].

- *De Quervains thyroiditis:*

The disease is known to occur usually following a recent upper respiratory tract infection or a viral syndrome. The thyroid gland

appears enlarged and is tender on palpation. According to Shabb et al; 1999, Garcia Solano et al; 1997 and Lowhagen and Willems; 1981, the aspirate typically shows benign looking follicular epithelial cells along with epithelioid type of cells and Langhans type of giant cells. The other inflammatory cells which can also be seen are lymphocytes, plasma cells and rarely few neutrophils[33].

- *Lymphocytic thyroiditis or Hashimotos disease:*

This is one of the most common thyroiditis which usually affects middle – aged women[33]. According to Marluce Bibbo, Hashimotos thyroiditis can be of three types depending on the various stages of the lesion. They are:

- Juvenile lymphocytic thyroiditis
- Hypertrophic lymphocytic thyroiditis
- Fibrous lymphocytic thyroiditis

Key features of juvenile lymphocytic thyroiditis[34]:

- Plenty of lymphocytes
- Small amount of colloid
- Follicular cells in varying number

It is important to note at this stage that Hurthle cells are absent or occur rarely. The lymphocytes show rope like detritus which is actually artifactual[36].

Key features of hypertrophic lymphocytic thyroiditis[34]:

- Plenty of lymphocytes and plasma cells are seen

- Colloid is usually scant
- Mixture of Hurthle cells and follicular cells are seen
- Other cells such as macrophages, multinucleated giant cells and epithelioid cells may be seen.

Psammoma bodies are commonly present in this stage of the lesion[37]

- *Fibrous lymphocytic thyroiditis:*

This type of thyroiditis occurs in elderly. Aspirated material will show only fibroblasts, lymphocytes, follicular cells and oncocyti ccells[38].

Riedel's struma:

It is asclerosing inflammatory disorder involving the thyroid gland. This type of reaction can also be seen in the retroperitoneum, mediastinum and also in the orbit. The consistency of this type of lesion is rubbery. The aspirate will be scant and may show few fibroblast like cells. The significance of this tumor is its resemblance to infiltrating carcinoma and hence should be differentiated from infiltrating carcinoma.

NEOPLASTIC THYROID DISEASES

Follicular Tumors :

In histology, adenoma shows follicles of varying sizes filled with colloid. Sometimes follicular adenoma can show nuclei with marked enlargement and hyperchromasia. Exceptionally, the lining epithelium of the follicles would show cells with colloid in the cytoplasm pushing the nuclei to the periphery giving the appearance of signet – ring adenoma.

It is difficult to distinguish between the benign follicular tumor and malignant follicular tumor based on cytology alone. Follicular adenomas are capsulated and show follicular cells but without any invasion.

The term atypical adenoma can be used to describe a lesion which is encapsulated, highly cellular with bizarre nuclei and few mitosis. This type of lesion is considered as encapsulated papillary carcinoma by some of the authors[39]while others consider it to be follicular carcinoma in situ[40] as there is absence of vascular and capsular invasion which is typical of follicular carcinoma.

The difference between follicular adenoma and carcinoma is very subtle and can be differentiated only by the presence of invasion in to the tumor capsule, blood vessel adjacent to thyroid or distant metastasis[41] . In cytology, certain points should be considered before looking in to the cellular and architectural aspects for effective reporting. Some of the points to be noted are:

- A definitive diagnostic criterion cannot be derived based on cellularity of the smear per se. For example, a diagnosis of follicular neoplasm cannot be made based on the hypercellularity of the smear alone as this can also be noted in hyperplastic nodules.
- Most of the cytological features tend to overlap between certain conditions like nodular goitre, follicular adenoma and follicular carcinoma. Hence, the cytological categories which can be considered are “follicular neoplasm” or “follicular proliferation are not sharply defined”.
- He also stressed that various cytologic criteria should be brought into consideration before making a definitive cytologic diagnosis.

Key features of follicular neoplasm in cytology[34] are:

- The aspirate is haemorrhagic with little or no colloid
- The smear will show numerous clusters. These clusters are arranged in microfollicular pattern and as three dimensional clusters. Rarely rosetting and trabecular pattern can also be seen.

The background of the smear shows individual follicular cells and naked nuclei. The follicular cells may exhibit anisonucleosis with uniform chromatin and inconspicuous nucleoli. Aspirates from follicular carcinoma are also highly cellular and exhibit same cytologic features as that of follicular adenoma[33].

In a well differentiated follicular carcinoma, it is not possible to distinguish between adenoma and carcinoma. Hence, a diagnosis of "follicular neoplasm" is recommended suggesting follow up by excision and histopathological evaluation.

The less well differentiated form of follicular carcinoma may show nuclear atypia characterised by anisonucleosis, hyperchromasia and less commonly nuclear pallor and intracytoplasmic nuclear inclusions. Evident nucleoli can also be seen. Even in these cases with evident atypical nuclear features a diagnosis of "follicular neoplasm – excision recommended" is given[33].

Hurthle cell tumors:

Hurthle cell tumors in histology are encapsulated tumors which are composed of sheets and follicles of Hurthle cells with large, eosinophilic cytoplasm. The cytoplasm is usually granular[33].Hurthlecell acquires its morphology due to the accumulation of mitochondria in the cytoplasm[42]. Cytoplasm of these tumors also shows lumina within the cytoplasm which contains thyroglobulin. Colloid is usually scant or absent. The nuclei are usually large and irregular exhibiting hyperchromasia.

Rarely, Hurthle cell tumor becomes malignant by invading the capsule, blood vessels or adjacent organs. It is worth noting that there is a greater possibility of Hurthle cell tumor turning malignant than a follicular tumor[43].

Key features of Hurthle cell neoplasm in cytology[34]:

- The aspirate is cellular but the amount of colloid is minimal or absent.

- The oncocytic cells are seen predominantly in cohesive clusters and rarely scattered singly
- The cells are usually large and polygonal with eosinophilic granular cytoplasm. The nuclei may show prominent nucleoli.
- Background is usually devoid of lymphocytic infiltrates and cellular debris
- Prominent vascularity can be noted in most of the cases.

The smear usually appears monomorphic as most of the cell population is replaced by these oncocytic cells leaving behind only a few or no normal looking follicular cells[33].

Hurthle cells can also exhibit a papillary pattern but without the typical nuclear features of papillary carcinoma thyroid[44].

According to Silver and Busseniers, Hurthle cell neoplasm characteristically had prominent vascularity and intracytoplasmic lumina[45]. This report was reviewed by Das et al. and he reported that, presence of transgressing vasculature but not intracytoplasmic lumina is characteristic of Hurthle cell neoplasm.

The distinction between benign and malignant condition is difficult and the diagnostic precision is as low as 60%[46] using various criteria. Renshaw later derived five cytologic criteria which could be more specific for malignancy in Hurthle cell neoplasm[34].

- Smears should have predominantly Hurthle cells and absent or scant colloid
- Cells with cytoplasmic diameter which are two times lesser than the nuclear diameter
- Large cell dysplasia
- Crowding of the cells with the nuclei touching each other
- Oncocytic cells arranged singly

Skoog and Tani suggested that, Ki-67 proliferation index along with the above said findings can increase the chance of detecting Hurthle cell malignancy as, Hurthle cell malignant tumors have a three-fold higher proliferation index than the adenomas[48].

It is also noted that Hurthle cell tumors can produce central ischaemic necrosis following fine needle aspiration. This phenomenon is not seen with any other thyroid neoplasm[48].

Medullary carcinoma:

Medullary carcinoma tends to derive from C cells. The tumor can occur denovo or may occur as a component of familial multiple endocrine neoplasia (MEN). The genesis of this tumor rests on the mutation of RET proto - oncogene.Histologically, medullary carcinoma may be single or multiple. The neoplastic cells are seen to be arranged in sheets and nests like pattern. The epithelial tumor cells may be of different sizes. These cells are large polygonal

with neuroendocrine granules in the cytoplasm which are diagnostic of medullary carcinoma[35].

Key features of medullary carcinoma in cytology[34]:

- Smears show plenty of blood with epithelial cells arranged loosely in mono layered sheets.
- The cells may be round, polygonal, plasmacytoid, polygonal or spindled.
- The cytoplasm is dense and shows metachromatic granules.
- Nuclei can be oval or pleomorphic with granular chromatin and few small nucleoli
- Naked nuclei can be noted in the background
- Some cases show dense amorphous material which is characteristic of amyloid.

The cytoplasmic granules are not considered pathognomonic as they can be seen in other conditions like follicular tumor, anaplastic carcinoma and metaplastic carcinoma of breast. It is also noteworthy to state that these granules cannot be seen in all cases or in all cells[49].

Anaplastic carcinoma:

This is a highly malignant tumor usually occurring in old age.

Histologically, This tumor is usually seen to infiltrate the thyroid gland and adjacent neck structures at the time of diagnosis. The anaplastic carcinoma may be of two types; spindle and giant cell carcinoma and a small cell type carcinoma[33].

Cytologically, Key features of anaplastic carcinoma are[34]:

- Smears show isolated tumor cell clusters forming three dimensional groups
- The background of the smears will be haemorrhagic and show extensive necrosis.
- The cells will appear bizarre exhibiting varying cell morphology with pleomorphic nuclei and prominent nucleoli.

The cells are giant or mixed cell type. The cytoplasm can appear densely granular or may sometimes contain small vacuoles. The shape of the nucleus may vary from round to oval exhibiting irregular contour. Frequent atypical mitosis may be observed. Background may also show inflammatory infiltrates rich in neutrophils. Multinucleated giant cells may also be noted[34].

Papillary carcinoma:

The commonest among the tumors of thyroid is papillary carcinoma (80% or even more)[50].Clinically they present as a palpable thyroid nodule.

- *Epidemiology:*

The prevalence of this tumor is common among women with female to male ratio of 4:1. Most of these tumors manifest between the age group of 20 – 50 years. In more than half of the diagnosed patients, the cervical lymph nodes are involved at the time of surgery. The survival with papillary carcinoma is excellent (>90%) in the younger patients[50].

- *Clinical features:*

Papillary carcinoma usually presents as a thyroid mass or thyroid nodule. In areas of iodine insufficiency, papillary carcinoma may arise as a distinctive nodule in multinodular goitre whereas, in areas with adequate iodine, they present as a solitary nodule in an otherwise normal thyroid gland[50].

These nodules do not absorb radioactive iodine and is therefore cold on scinti-scan, presenting with or without palpable lymph nodes. In more than 20% of these patients, initial finding may be metastatic involvement of the cervical lymph node with no palpable neck lesion or mass[51].

Certain incidental, non-palpable thyroid nodules can show microscopic foci of papillary carcinoma. The clinical significance of this incidental finding is negligible as younger patients with even larger masses can have a 20 years survival[50].

- *Thyroid function test:*

The presence of thyroid malignancy does not alter the functional capacity of the thyroid gland. Hence, thyroid function test does not aid in the diagnosis of papillary carcinoma thyroid. This test can be done to evaluate the functional capacity of the gland[50].

- *Imaging:*

Some of the diagnostic modalities which can be useful are ultrasonography, radio–active iodine scans, computerized tomography scans and magnetic resonance imaging. These modalities are required only in older patients with definitive symptoms of malignancy and local tissue involvement to define the extent of tumor involvement[50].

- *Gross Finding:*

Variety of patterns can be noted grossly in papillary carcinoma of thyroid. The cut surface usually appears firm, grey white and with irregular borders seen to involve and extend in to the surrounding parenchyma.

Some of the degenerative changes which can be noted are dystrophic calcification and rarely cystic changes. Rarely, primary tumors appear solid but the nodal metastasis may be cystic.

Tumors can also arise in thyroglossal duct cysts. A direct extension in to adjacent tissue such as

peri-thyroid fat, skeletal muscle, oesophagus, larynx and trachea can also be noted[50].

- *Tumor spread and staging:*

Papillary carcinoma has a high tendency for lymphatic dissemination. Hence, regional lymph nodes are involved in most of the cases. Rarely, intrathyroid lymphatic spread can also occur, leading on to separate tumor nodules with in the thyroid.

Staging of the tumor relies on age of the patient, size of the primary tumor, extrathyroidal spread, regional and distant metastasis[50].

- *Histopathology:*

Papillary carcinoma microscopically has a papillary architecture which is typically complex and shows branching pattern. These papillae are lined by epithelium with cells showing pale to eosinophilic cytoplasm and nuclei exhibiting loss of polarity. The most striking finding in papillary carcinoma is its nuclear features which are diagnostic even in the absence of papillary architecture.

The characteristic nuclear features include change in size and shape such as nuclear enlargement, nuclear elongation, oval nuclei and overlapping of nuclei. The nuclei typically show clearing with irregular nuclear contour. Nuclear grooves and pseudo inclusions are also commonly seen. The presence

of above said nuclear features in significant proportion is diagnostic of papillary carcinoma of thyroid.

Extensive squamous metaplasia lining the cyst wall is seen. Psammoma bodies which are rounded and concentrically laminated calcifications can be seen with in the lymphatic spaces or anywhere within the tumor stroma. Sclerosis can also be noted with in the tumor[50].

Histopathological variants of papillary carcinoma thyroid according to WHO 2003 :

- *Follicular variant:*

Grossly, these tumors are encapsulated and have an appearance similar to that of follicular neoplasm.

Microscopically, these tumors will be composed of small follicles as in follicular neoplasm and usually with no evident papillary structure. These tumors are identified as papillary carcinoma based on the classical nuclear features such as nuclear grooves and pseudoinclusions.

Other associated rare findings are intrafollicular multinucleated giant cells, stromal sclerosis and psammoma bodies.

Cytologically, the diagnosis is difficult as the nuclear features are not clearly evident[52]. They are characteristically seen in flat sheets and may occasionally exhibit branching and crowding of the nucleus.

According to a study conducted by Shyang- Rong Shih and team on the sensitivity and specificity of FNAC on follicular variant of papillary carcinoma thyroid, they concluded that FNAC may be a good tool to diagnose papillary carcinoma thyroid but is unreliable to differentiate follicular variant and usual variant of papillary carcinoma of thyroid[53].

- *Macro follicular variant:*

This is the rarest form of papillary carcinoma. This variant shows only macrofollicles or a minimum of 50% cross sectional area show only macrofollicles. This type of variant is confused with colloid nodule as most of these tumors are encapsulated.

The defining feature is its characteristic nuclear changes such as nuclear grooves and pseudoinclusions[50].

- *Oncocytic variant*:

Grossly this type of tumor shows a distinct mahagany brown appearance. Rarely cut surface appears grey white.

Microscopically, these tumors show papilla with thin fibrovascular core which is characteristically lined by oncocytic cells. This type of variant is commonly associated with Hashimotos thyroiditis. The oncocytic cells are usually polygonal and rarely columnar with abundant eosinophilic cytoplasm. The

diagnosis is made based on the nuclear features. Careful examination is made to look for any invasion[50].

Cytologically, nuclei will show characteristic features of papillary carcinoma thyroid. The distinguishing feature of this variant is the presence of granularity in the cytoplasm[34].

- *Clear cell variant*:

This is a type of variant where conventional and follicular variants are composed predominantly of clear cells. In some case these clear cells are seen to be admixed with oncocytic cells. The nuclei will show features of conventional papillary carcinoma. Rare cases may show intra and extra cellular mucin.

- *Diffuse sclerosing variant:*

This is a tumor of the young with uniform involvement of both the lobes without any nodule formation. These tumors exhibit squamous metaplasia with psammoma bodies, fibrosis and lymphocytic infiltration. The non-tumorous counterpart usually shows chronic lymphocytic thyroiditis[50].

Cytologically, aspirated smear may show abundant lymphocytic inflammatory cells along with metaplastic squamous cells arranged in monolayered sheets and numerous psammomabodies[34].

- *Tall cell variant:*

Tall cell variant is a tumor of the old age, with male preponderance.

This is a rare variant in which the follicular cells have a height three times that of the width. The pattern of arrangement of the neoplastic cells is a combination of papillary structure, trabeculae and cord like, while follicular pattern is rare.

The neoplastic cells have abundant eosinophilic cytoplasm with nuclear features of conventional papillary carcinoma. Necrosis, increased mitotic activity and extra-thyroidal extension are common[52].

Cytologically, papillary structures with classical nuclear features are seen. The cells may appear tall with distinct borders, eosinophilic cytoplasm and a high nuclear: cytoplasmic ratio. The nuclei can show multiple inclusions giving a soap bubble appearance[54].

This type of variant can be confused with Hurthle cell neoplasm but is differentiated by the typical nuclear features of papillary carcinoma[34].

- *Columnar cell variant:*

This type of variant is composed of columnar cells exhibiting pseudostratification. They exhibit subnuclear or supranuclear vacuolation resembling those of secretory endometrium. The nuclei are predominantly hyperchromatic with few cells showing the typical nuclear features of

conventional papillary carcinoma. Problem arises in diagnosis when this type of variant is seen in metastatic deposits where the tumor can be misdiagnosed as a tumor of Gastro-intestinal tract or of the lungs. Columnar cell variant of papillary carcinoma can be differentiated by its immune reactivity with thyroglobulin and TTF–1.

They tend to be more aggressive than the conventional variant exhibiting extensive extra-thyroidal extension[50].

Cytologically, they resemble metastatic carcinoma of the ovary or large bowel[34]. They predominantly show papillary fragments lined by pseudostratified columnar epithelium with basally placed nuclei and stippled chromatin. The characteristic nuclear grooves and pseudoinclusions are infrequent and almost absent[55].

- *Solid variant:*

This type of carcinoma is common in children. They do not exhibit any pattern and are seen to be arranged in solid sheets with characteristic nuclear features of conventional papillary carcinoma[50].

- *Cribriform variant:*

This type of variant is common in patients with Familial Adenomatous Polyposis and Gardner's syndrome. In WHO classification of thyroid tumors,

this is considered as a variant of papillary carcinoma whereas other classifications consider this type separately.

This type of lesion is characterised by focal papillary architecture along with cribriforming, solid areas, spindled areas and formation of squamoidmorules. The typical nuclear features of papillary carcinoma are rarely seen and commonly the nuclei are hyperchromatic[50].

Cytologically, the cells are round to spindle and are seen to be arranged in sheets, follicular, cribriform or whorl like patterns. Scattered intra-nuclear cytoplasmic inclusions and grooved nuclei can be seen[45].

- *Papillary microcarcinoma:*

This term is used when the papillary carcinoma is an incidental finding and measures less than one centimetre in diameter. According to WHO 2003, it is the most common form of papillary carcinoma. This type of tumor can have aggressive course in children[50].

Their commonest location is adjacent to the capsule of the thyroid and the tumour is usually non encapsulated and sclerosing. These tumors can be very small measuring less than 1 mm with only follicular pattern or may be larger measuring about 2 mm and exhibit desmoplasia. Rarely these tumors can also exhibit papillary pattern[51].

Cytologically, papillary microcarcinoma is missed during regular FNA. When material is obtained, the cells would show typical nuclear features of papillary carcinoma along with some normal follicular cells and clear colloid[35].

Some of the other variants observed are "papillary carcinoma with fasciitis – like stroma", "papillary carcinoma with focal insular component", "papillary carcinoma with squamous cell or mucoepidermoid carcinoma", "papillary carcinoma with spindle and giant cell carcinoma" and "combined papillary and medullary carcinoma".

- *Genetics:*

The most common genetic alteration noted in papillary carcinoma thyroid is RET gene rearrangement called RET/PTC. Other mutations which can be encountered are TRK rearrangements, RAS mutations, BRAF mutations and mutation of CTNNB 1 gene encoding the β catenin gene. RET/PTC rearrangements are specific for early events in the development of papillary carcinoma of thyroid[43].

Fine needle aspiration cytology[51]:

- In any solitary thyroid nodule, cytologic analysis should be done first which will help 75% of people with benign cytology from further studies and surgery.
- Papillary carcinoma can be effectively diagnosed by FNAC

- Metastatic deposits in the cervical lymphnodes can also be diagnosed effectively by FNAC.

  - *Diagnostic Pitfalls*[31]*:*

- In some cases confusion in diagnosis results due to artifact caused by air-drying of FNA specimens. This can lead to enlargement and hyperchromasia of the follicular cells. The critical part is that, the chromatin pattern can acquire a round shape with sharp borders and can closely resemble intra nuclear cytoplasmic inclusions of papillary carcinoma. This type of pseudo-clearing can be accentuated by the presence of large amount of peripheral blood which in turn can interfere with the fixation of smear. This error can be prevented by using a thinner gauge needle (preferably, a 25-gauge needle) and by limiting the number of passes.
- In diagnosing a papillary carcinoma, frozen section has its limitations owing to the loss of nuclear details due to freezing artefact. Freezing can lead to artifactual nuclear clearing, which can be mistaken for papillary carcinoma. Hence, an adjunct intraoperative cytologic study can help to avoid false positive diagnosis of papillary carcinoma in frozen section.

- In routine practice it is difficult to differentiate between reactive changes associated with chronic lymphocytic thyroiditis and papillary carcinoma thyroid arising in the background of chronic lymphocytic thyroiditis as follicular epithelium adjacent to lymphoid aggregates can show nuclear changes such as chromatin clearing, pseudoinclusions and grooves similar to that of papillary carcinoma thyroid.
- Hurthle cell lesions can be mistaken for Warthin-like papillary thyroid carcinoma as few of the Hurthle cells may show nuclear grooves. This can be differentiated from papillary carcinoma as these lesions lack other nuclear features of papillary carcinoma.
- Hyperplastic ultimobranchial body rests can demonstrate nuclear chromatin clearing and grooving but these lesions can be differentiated from papillary carcinoma as their location is in the lateral lobes. In cytology, ultimobranchial body appears as a monotonous population of small cells and these solid cell nests stain positively for cytokeratin and exhibit negativity for thyroglobulin and thyroid transcription factor-1.
- Solitary papillary hyperplastic nodules can be sometimes mislabelled as "suspicious of" or consistent with papillary carcinoma. Cytologically, they can be differentiated by the presence of flame

cells, watery colloid, lack of pseudoinclusions and presence of non-branching papilla.

- Graves disease can also exhibit nuclear papillary hyperplasia and can be mistaken for papillary carcinoma. In FNA specimen, nuclear atypia can occur as nuclear enlargement along with hyperchromasia or nuclear chromatin clearing and hence it is difficult to make a diagnosis of papillary carcinoma thyroid in a gland affected with Graves disease. While reporting papillary carcinoma in a case of Graves disease, care should be taken that all the nuclear features are present before reporting.

According to Moira D. Wood, presence of three variables namely anisokaryosis, nuclear overlap and scant or absent colloid in cases of cellular smears should raise the suspicion of malignancy[56]

According to Bethesda system for reporting thyroid cytopathology (Springer 2010), four patterns are described to label a thyroid nodule as suspicious for malignancy:

Pattern A (Patchy nuclear changes pattern)

- Moderate or high cellularity
- Benign looking follicular cells arranged mostly in macrofollicle pattern
- Nucleus showing grooves, membrane irregularity or moulding.

- Rare or absent Intra Nuclear Cytoplasmic Inclusions (INCIs)

Pattern B (Incomplete nuclear changes pattern)

- Cellularity can be variable
- Nuclei may exhibit mild to moderate enlargement
- Nuclear grooves are evident but other features such as nuclear irregularity and nuclear moulding may be absent
- Intranuclear pseudoinclusions are absent
- *Pattern C (Sparsely cellular specimen pattern)*
- Most of the nuclear features of conventional papillary carcinoma are present but the cellularity is very sparse.

Pattern D (Cystic degeneration pattern)

- This type of pattern shows cystic degenerative changes which are evidenced by the presence of hemosiderin laden macrophages
- Smears show follicular cells in sheets and occasionally in groups
- The nuclei of these follicular cells have enlarged nuclei, some of them exhibiting nuclear grooves but pseudoinclusions are rare
- Occasionally, histiocytoid type of cells with enlarged nuclei and abundant vacuolated cytoplasm can be noted.
- Rarely calcification resembling psammoma bodies are noted.

According to a retrospective study conducted by Lisset Castro- Gomez, benign thyroid cysts and cysts of papillary carcinoma thyroid can be differentiated by the presence of certain features such as tri-dimensional fragments, anisonucleosis, grooves, pseudoinclusions, metaplastic cytoplasm and powdery chromatin in the latter[58].

In another study conducted by William C. Faquin and colleagues, they studied FNA smears of cystic lesions of thyroid and looked for cytologic and corresponding histologic features of atypical cells in thyroid cysts and concluded that atypical cells with characteristic features of distinct cell borders, elongated shape, eosinophilic cytoplasm and distinct nucleoli but lacking nuclear crowding, pseudoinclusions and papillary structure which is characteristic of papillary carcinoma thyroid should be reported as " consistent with benign cyst lining cells"[32].

In 2005, Sanjay Jogai and colleagues did a study to analyse the diagnostic efficiency of fine needle aspiration cytology to evaluate thyroid nodules with special consideration on cytologic diagnosis with discrepancy. In their study they considered four different categories of cytologic diagnosis namely positive for malignancy, negative for malignancy, indeterminate for diagnosis and non-diagnostic. All the cases were later followed up with histopathologic diagnosis. In their conclusion, they have mentioned that FNAC is a safe, sensitive and specific procedure for the primary evaluation of thyroid nodules.

They have also mentioned that in most of the cases, cytological analysis is helpful to arrive at a correct diagnosis and thus directing towards the need for secondary surgical intervention.

They concluded that improper sampling can lead to false negative diagnosis and over-interpretation of cytologic findings can result in false positive diagnosis[59].

Criteria for papillary carcinoma of thyroid (conventional and variants) according to Bethesda System:

- Follicular cells may be arranged in papilla and/or in monolayered sheets.

The nuclear changes that can be seen in these follicular cells are[57]:

- Enlarged nuclei which may be oval or sometimes irregular in shape
- Longitudinal nuclear grooves
- Intra Nuclear Cytoplasmic Inclusions (INCI)
- Pale nuclei with powdery chromatin
- Marginally placed micronucleoli
- Psammoma bodies
- Multinucleated giant cells
- Ropy or bubble gum like colloid

- Oncocytic metaplasia
- Squamous metaplasia

Among the above mentioned nuclear features, none is specific or diagnostic for papillary carcinoma thyroid when present as an individual feature or when present in low quantity. The diagnosis of papillary carcinoma is given based on combination of some of the above mentioned features.

In our study we have selected five commonly occurring features of papillary carcinoma and tried to evaluate the occurrence of each of these feature in papillary carcinoma as well as in other thyroid swellings, thereby identifying their significance in the diagnosis of papillary carcinoma.

The five features which we selected for our study are;

- Nuclear Grooves
- Pseudoinclusions or Intra Nuclear Cytoplasmic Inclusions (INCIs)
- Papillary Fragments
- Metaplastic Cytoplasm
- Three Dimensional Fragments

- *Nuclear Grooves:*

Grooves are due to the infolding of nuclear membrane and they appear as multiple longitudinal furrows or superficial notches resulting in lobed appearance of the nucleus. These grooves are usually oriented parallel to the long axis of the oval shaped nuclei giving a coffee bean appearance[57].

The grooves are easily appreciated in ethanol fixed and Papanicolaou stained smears. Grooves are easy to identify in individually scattered cells than tissue fragments as nuclear overlap can mimic them.

A study conducted by Anita Tahlan and team found that nuclear grooves are not specific for papillary carcinoma thyroid but they could be found in other non - neoplastic conditions. They concluded by stressing that nuclear grooves should be given appropriate weightage for the diagnosis of malignant lesions of thyroid[60].

Some of the other diseases which can present with occasional grooving are[57]:

- Follicular Adenoma
- Follicular Carcinoma
- Nodular Goitre
- Hashimotos Thyroiditis
- Rarely Metastatic Tumors (Melanoma, Carcinoma)

According to Yi Jun Yang MD, a semi-quantitative approach could be helpful in quantifying the nuclear grooves and thereby help in the diagnosis of papillary carcinoma when the other diagnostic features are absent. They concluded that presence of more than or equal to 20% nuclear grooves is virtually diagnostic of a neoplasm, most likely papillary carcinoma[61].

- *Intra Nuclear Cytoplasmic Inclusions (pseudoinclusions):*

Pseudo inclusions are present in more than or equal to 5% ofcells in 90% of the cases[62][63] . Pseudoinclusions are finger like invagination of cytoplasm in to the nucleus.

Characteristic cytologic features of pseudoinclusionare[57]:

- They are membrane bound
- Large and occupy more than 50% of the nuclear area
- They have tinctorial pattern similar to cytoplasm
- Optically more clear than surrounding chromatin
- They tend to have outside rim of condensed chromatin

It is noteworthy to state that optically clear nuclear area containing biotin and air-dried or ethanol fixed papanicalou stain can simulate intra nuclear cytoplasmic inclusion and care should be taken to differentiate this from the artefacts.

Other diseases that can present with Pseudoinclusions are[57]:

- Anaplastic Carcinoma

- Medullary Carcinoma
- Follicular Carcinoma
- Hurthle cell Carcinoma
- Metastatic RCC
- Hashimotos thyroiditis

According to some authors presence of more than three enlarged nuclei with pseudoinclusions is pathognomic of papillary carcinoma[64]

- *Papillary structure:*

In cytology, papillary structure can present with different morphologies and dimensions depending on the aspirate. If the papillae are completely aspirated they appear as large fragment with prominent vascular network. If they are partially aspirated they tend to appear in flat sheets and if the tip of the papillae is aspirated, they give the appearance of spherical cellular fragment exhibiting palisading and nuclear overlapping[57].

- *Squamous metaplasia*:

It is the waxy or squamoid quality exhibited by the cytoplasm of the malignant cells[57].

In 2007, Dana Weberconducted a different study, taking in to consideration, the presence of atypical epithelial cells in fine needle aspiration of thyroid. Their objective was that, the diagnosis of “atypical epithelial cells, cannot exclude papillary thyroid carcinoma” (AEC-PTC) in FNA is very controversial and this can lead to a dilemma in patient management. In their

study they included eighty-eight thyroid FNA specimens with this diagnosis. They found that one of the common reasons for giving a diagnosis of atypical cells in aspiration smear is because of the rare presence of atypical cells in a cystic nodule or the presence of atypical cells in a background of Hashimotos thyroiditis.

They concluded that, presence of atypical epithelial cells along with nuclear features such as INCI, squamoid cytoplasm and psammoma bodies should alert the pathologist to rule out the possibility of papillary carcinoma. They have also mentioned that, occurrence of focal cytological features of papillary carcinoma in fine needle aspiration samples are strongly associated with papillary carcinoma in the follow up resection[65].

Dilip K. Das and Prem N. Sharma conducted an intriguing study to evaluate the imperfection in making a decision of papillary carcinoma in fine needle aspiration smears. They considered five cytomorphological features comprising of papillary formation, intra-nuclear cytoplasmic inclusions, nuclear grooves, fine nuclear chromatin and psammoma bodies. The frequency of above mentioned nuclear features were analysed and scored. Their conclusion was that, presence of more than or equal to three of the above said five cytologic features helped to arrive at a definitive diagnosis of papillary carcinoma thyroid easily. They have also mentioned that, not just the mere presence of any of the three above said features but the number of cytomorphological features along with occurrence of certain specific features

and their extend in FNA smears account for the confidence of cytopathologists in making a diagnosis of papillary carcinoma thyroid[66].

A study was conducted by Mary K. Sidawy to evaluate the results of thyroid fine needle aspiration and to detect the reason for discrepancy of diagnosis between cytology and histopathology.They evaluated 133 FNA's, which also had a follow up histopathologic diagnosis. They concluded that, the discrepancy mainly occurred between anaplastic neoplasm, follicular neoplasm and follicular variant of papillary carcinoma because of their overlapping cytologic criteria[67].

Jack Yang and his colleagues conducted a study in 2007 with the background of six diagnostic categories proposed by Papanicolaou society of cytopathology for classifying thyroid fine needle aspiration cytology cases. Using these categories, the experience with FNA from two institutions was studied with emphasis on cyto-histologic correlation, source of errors and clinical management.

The six diagnostic criteria were classified as unsatisfactory, benign, atypical cellular lesion, follicular neoplasm, suspicious for malignancy and positive for malignancy.

From their results, they concluded that fine needle aspiration provides an accurate diagnosis of thyroid malignancy. All the above mentioned six diagnostic categories were beneficial for triaging patients for either clinical follow-up or surgical treatment[68].

In a study conducted by Andrew A. Renshaw, few cases with focal features of papillary carcinoma accounting up to less than 20% of the cells were selected and he concluded that most of these cases turned out to be follicular variant of papillary carcinoma in the follow-up histo-pathologic report[69].

MATERIALS AND METHODS

TYPE OF STUDY:

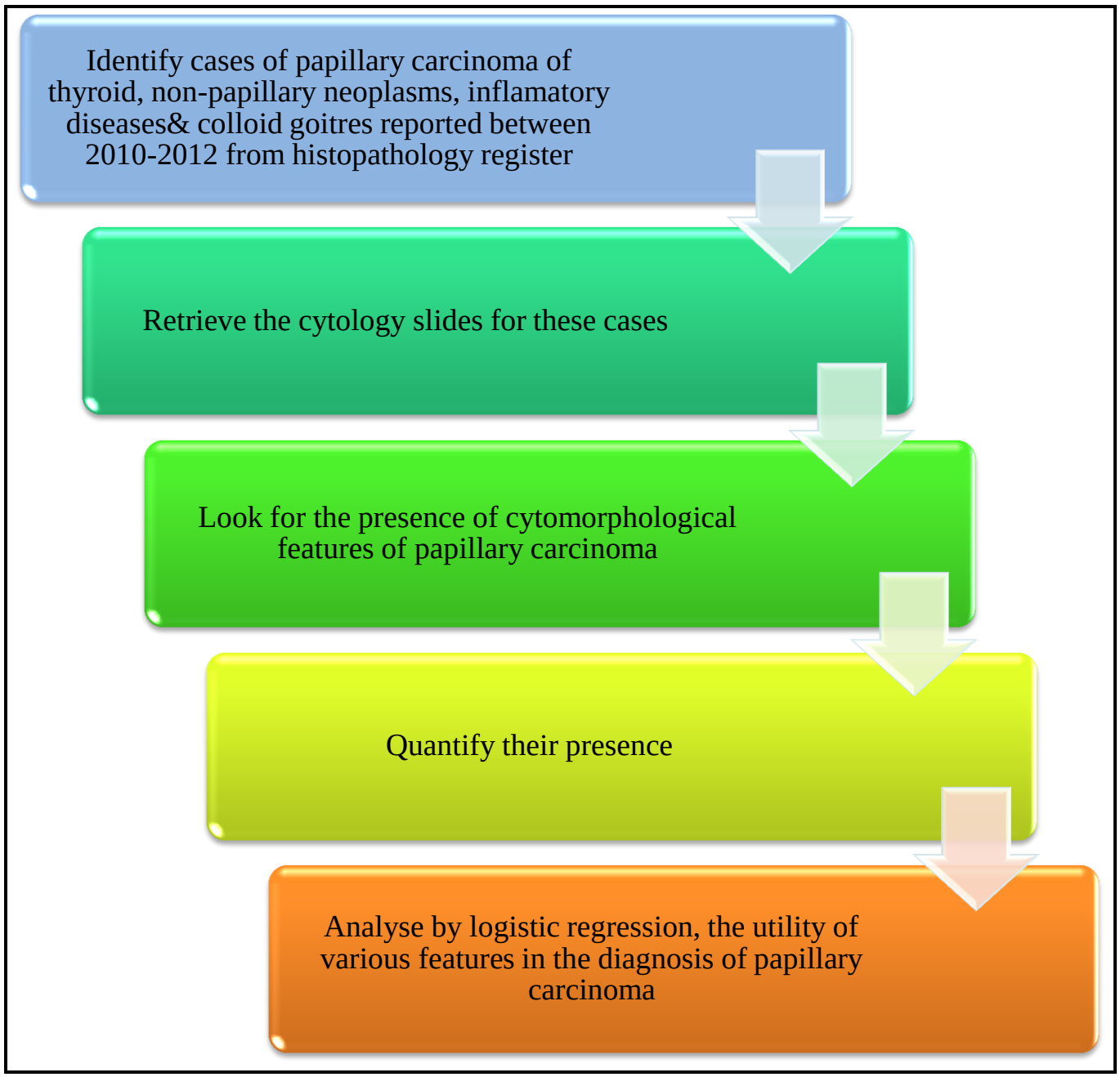
This is a retrospective study conducted on all thyroid FNA cases which had histopathology correlation between the years 2010 and 2012, in the department of Pathology, PSG Institute of Medical Sciences and Research.

STUDY DESIGN:

- The histopathology and cytology registers between 2010 and 2012 maintained in the department of pathology were used to collect all the thyroid cases reported between the above said years.Only those cases who had both histopathology and cytology diagnosis were considered for our study population.
- The cytology slides of the study population were retrieved
- All these slides were reviewed and observed for the presence of cytomorphological features selected for our study by me and later by my guide.
- While reviewing, all those cases with atleast one of the five cytomorphologic features were included in the study and those cases without any of the specified features, were excluded from the study.

- The other exclusion criteria considered were hypo cellularity of the smear and ill preserved smears hampering the view.

Flow Chart of Study Design:



The specimens were obtained using 23 – 25 gauge needles for aspiration. All the FNA cytology smears in our institution are stained routinely with Giemsa, Papanicolaou and Hematoxylin & Eosin.

PERFORMING THE ASPIRATION:

- The lesion to be aspirated is grasped with one hand, most often with two fingers.
- The skin is draped with an alcohol soaked cotton ball
- The syringe with attached needle is laid against the skin at a predetermined puncture site
- The needle is then inserted through the skin in to the immediate subcutaneous tissue, by a smooth but quick motion
- The needle is then advanced in to the mass and felt for any resistance in the capsule as the needle passes
- Suction is applied to the aspiration syringe
- With constant suction pressure, the needle is moved back and forth with in the tumor, using short, quick strokes. Simultaneously, the hub and the tip of the needle is looked for appearance of any specimen. It is to be noted that high quality smears are obtained by keeping the specimen with in the needle and aspirating excess blood or fluid will dilute the cellular component.

- Once the specimen appears in the hub, the negative pressure is released allowing the vacuum in the syringe to return to normal.
- The needle is now gently withdrawn from the mass
- Immediate pressure is applied over the aspirated site with a sterile gauze pad
- The needle should not be withdrawn from the lesion with any vacuum pressure in the syringe as the aspirated sample may be sucked up in to the syringe
- In case of cystic lesion, the cyst should be evacuated completely and FNA repeated if there is any residual mass

PREPARATION OF SMEAR:

- Immediately after aspiration, the needle is removed from the syringe and the air is sucked in to the syringe by pulling the piston
- The needle is then re attached and the tip of the needle is placed on the centre of the slide, so that the tip of the needle touches the slide
- The plunger of the slide is then advanced so that a small drop of the sample approximately measuring 1 – 3 mm in diameter is expressed on to the slide.
- Continue the above said procedure over a series of slides
- Another slide is inverted over the drop and spread to form a smear
- Above smearing is repeated for all the smears

- A few slides are immediately fixed in 95% alcohol
- The other unfixed smears are air dried
- Then the smears are labelled with a unique serial number (laboratory number)

PAPANICOLAOU STAIN:

It is a polychrome counter stain method, based on dye competition in the cytoplasm combined with a nuclear Hematoxylin stain.

✓ *Procedure:*

- The alcohol fixed smears are hydrated in descending grades of alcohol – 95% - 2 min, 80% - 3 min, 75% - 2 min
- Rinse in water for one minute
- Stain in Harris Hematoxylin for one min
- Blueing in water
- Two dips in acid alcohol for differentiation
- Wash in water
- Dehydrate in ascending grades of alcohol
- Stain in OG 6 for two min
- Rinse twice in 95% alcohol
- Stain in EA 50 solution
- Wash in alcohol
- Mount with DPX

After staining, the nuclei will appear blue/black and cytoplasm will appear green.

WRIGHT – GIEMSA STAIN:

The stain consists of Methylene Blue / Azure B and Eosin in Acetone free Ethanol.

Giemsa stains are specific for phosphate groups and binds to regions with high amounts of adenine – thymic bonding.The cellular details are identified better.

- ✓ *Procedure:*
  - The air dried unfixed smears are fixed in methanol for five minutes
  - Allow to dry for required time
  - Add one part Giemsa and three parts distilled water and wait for twenty minutes
  - Wash and air dry
  - Mount with xylene.

The nuclei will appear purple / blue, cytoplasm pink / blue and the eosinophil pink / red.

SCORING:

The scoring for each of the nuclear features is different and was done based on the following criteria:

Nuclear grooves:

For a given case, all the slides retrieved were reviewed and representative slides with best cellularity were selected. These selected slides were then screened meticulously and fields and cell clusters with evident and frequently occurring nuclear grooves were identified. While selecting clusters for counting nuclear grooves, clusters with cells exhibiting oval nuclei were selected as they show more grooves than the other cells with regular round nuclei. Thus, by applying these stringent criteria, counting of nuclear grooves were done in five hundred cells and average percentage was taken for scoring.

Pseudoinclusions:

The representative slides and the clusters of follicular cells were selected in the same way as done for the nuclear grooves. They were screened for intra nuclear cytoplasmic inclusions in five random HPF in the clusters where they occurred most frequently. An average percentage was taken for scoring.

If the percentage of cells with pseudoinclusions was more than or equal to 5%, the occurrence was called as frequent and if it was less than 5%, it was called as infrequent pseudoinclusions.

Papillary fragments:

All the cases were reviewed for presence of atleast one papillary fragment in any of the slides. Accordingly, the findings were noted as present or absent.

Metaplastic Cytoplasm:

All the cases were reviewed for presence of cells with metaplastic cytoplasm. They were designated as positive for metaplasia, if any of the cells exhibited the same and negative if there was absence of such cells.

Three Dimensional Fragments:

All the cases were reviewed and presence or absence of three dimensional fragments in the slides was noted as present or absent TDF.

MASTERCHART:

A master chart was prepared with the following parameters;

a. Cytology lab. number and diagnosis
b. Histopathology lab. number and diagnosis
c. Nuclear grooves percentage
d. Pseudo inclusions percentage
e. Papillary Fragments – present/absent
f. Metaplastic cytoplasm - present/absent
g. Three Dimensional Fragments - present/absent

The information regarding the cytology and histopathology lab numbers and diagnosis were collected from the registers maintained in the department of Pathology, PSG Institute of Medical Sciences and Research.

Our study was based on the five parameters mentionedfrom (c) to (g).

DATA ANALYSIS:

Separate tables were made for all the five features.

Nuclear grooves:

Many methods have been followed worldwide (as given in literature) in categorising and quantifying the nuclear grooves. In our study we followed a quantitative approach for categorising nuclear grooves.

All the cases with presence of nuclear grooves were divided in to three groups based on the percentage of grooves

- ≥ 20% cells with grooves
- 10 – 19% cells with grooves
- < 10% cells with grooves

Intra Nuclear Cytoplasmic Inclusions:

In our study, we followed a semi quantitative approach to assess the frequency of intra nuclear cytoplasmic inclusions. All the cases with pseudo-inclusions were divided in to three groups as

- cells with frequent pseudoinclusions (≥5%)
- cells with infrequent pseudoinclusions(<5%)
- absent pseudoinclusions

For evaluating the other three features, the cases were divided into two groups, based on the presence or absence of that specific feature.

The diagnostic specificity, sensitivity, positive predictive value and negative predictive value of nuclear grooves, intranuclear cytoplasmic inclusions, papillary fragments, metaplastic cytoplasm and three dimensional fragments for the diagnosis of papillary carcinoma were determined by the following statistical analysis.

STATISTICAL ANALYSIS:

Diagnostic test evaluation analysis of all the five features i.e. Nuclear grooves, Pseudo inclusions, Papillary fragments, Metaplastic cytoplasm and Three-dimensional fragments was done using Medcalc statistical software. Statistical analysis included:

1. Sensitivity (probability that a test result will be positive when the disease is present – 'true positive rate')
2. Specificity (probability that a test result will be negative when the disease is not present – 'true negative rate')
3. Positive predictive value (probability that the disease is present when the test is positive)
4. Negative predictive value (probability that the disease is not present when the test is negative)

We used the following table to derive the above said data.

	Test		
Disease	0	1	Total
0	True Negative	False Positive	X
1	False Negative	True Positive	Y
Total	P	Q	Z

Dis 0 & test 0 = True negative

Dis 0 & test 1 = False Positive

Dis 1 & test 0 = False Negative

Dis 1 & test 1 = True Positive

Sensitivity = True positive / (True positive + False negative)

Specificity = True negative / (True negative + False positive)

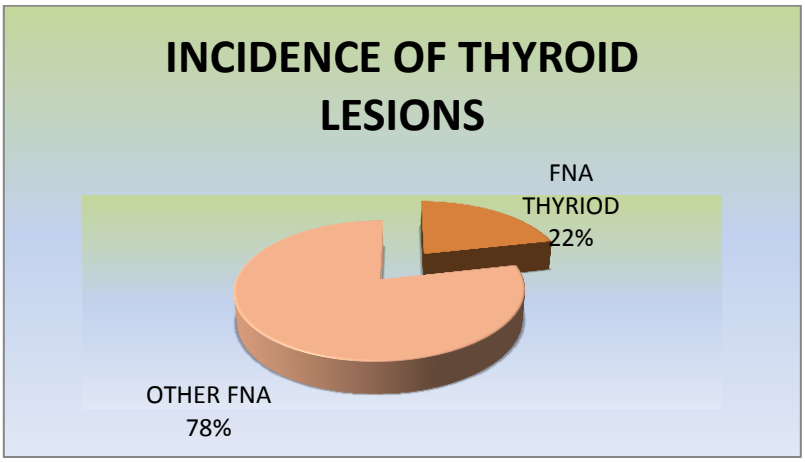
Positive predictive value = True positive / (True positive + False positive)

Negative predictive value = True negative / (True negative + False negative)

RESULTS

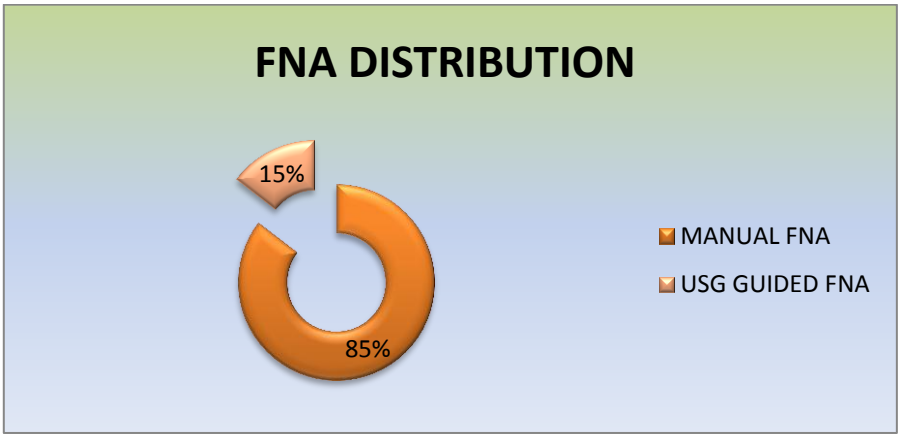
The period of study was from 1st January 2010 to 31st December 2012. Total numbers of FNAs done during this study period were 2583 and average FNAs done per year was approximately 861. Among the 2583 cases, thyroid FNA done during this study period was 556 (22 %)*(fig1)*

Figure 1: Incidence of Thyroid Lesions



Among the 556 FNA performed, 475 cases (85%) were manually aspirated and the remaining 15% were done under the ultrasonography guidance *(Fig 2)*

Figure 2: FNA Distribution



The occurrences of thyroid lesions were commonly noted between 3rd and 5th decade *(figure 3)*. Females were more commonly affected and comprised 87% of study population in comparison to males accounting for 13% of the study population *(figure 4)*.

Figure 3: Age Distribution

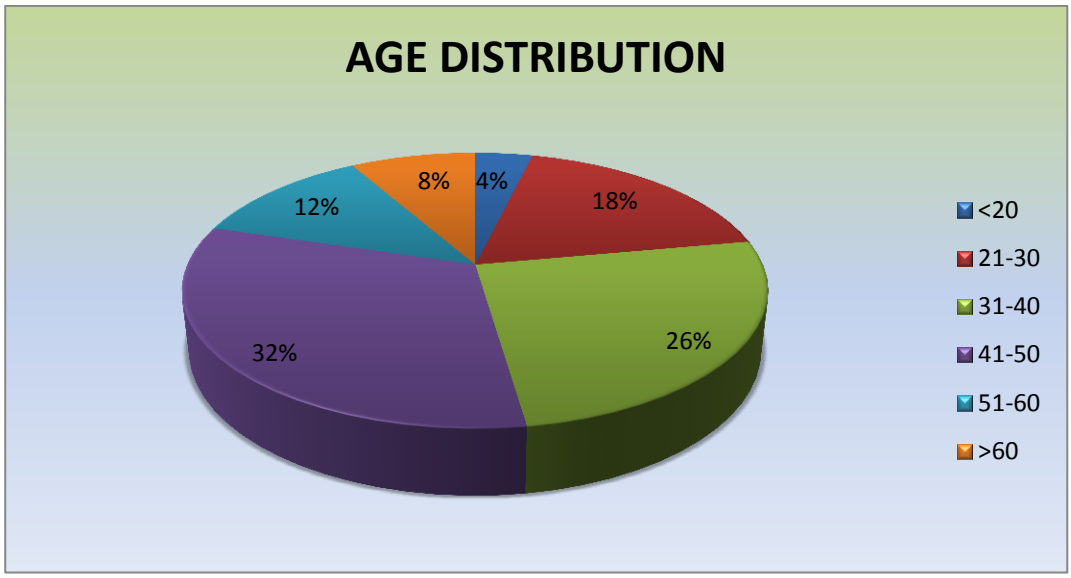
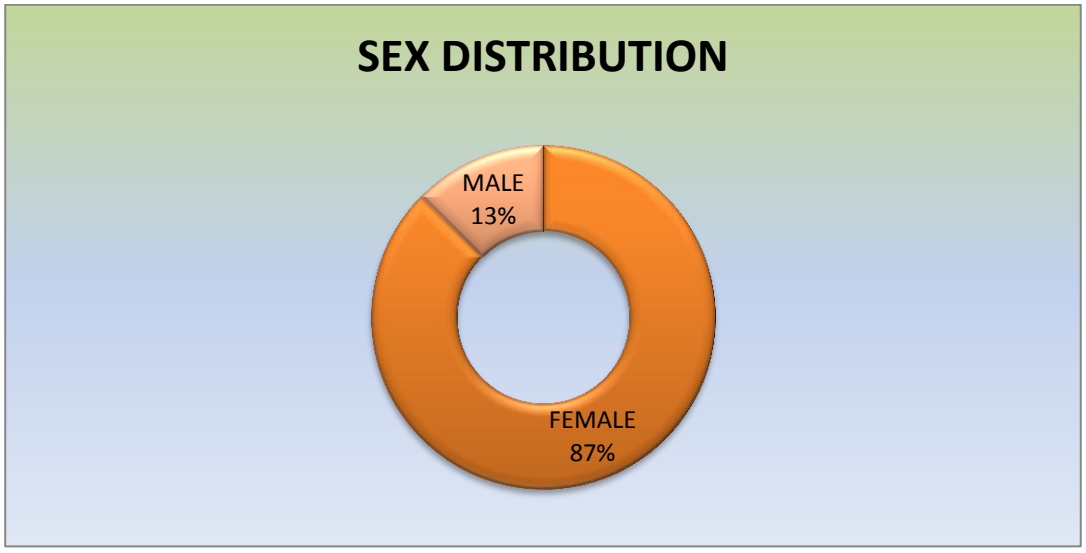
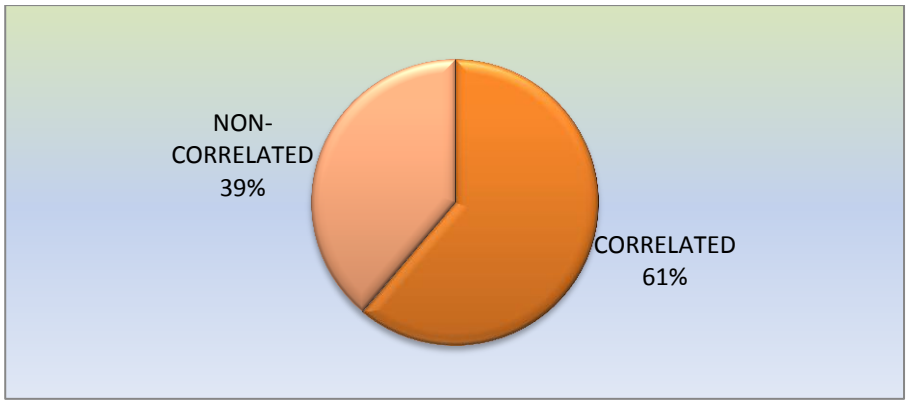


Figure 4: Sex Distribution



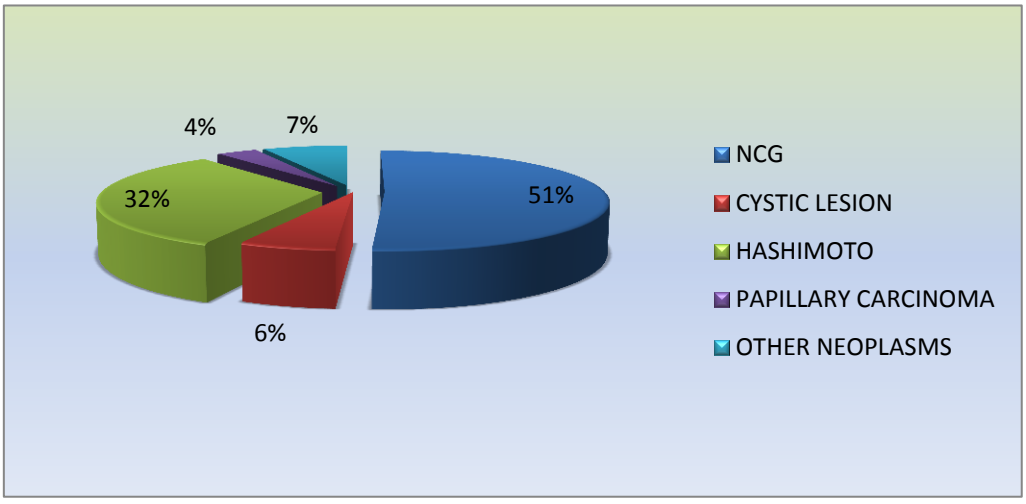
The correlation between cytology and histopathology diagnosis during the study period was 61% *(figure 5)*

Figure 5: Cytology – Histopathology Correlation



The distribution of diseases by FNA diagnosis in adequately sampled cases during the study period were 254 (51%) cases of nodular colloid goitre, 160 (32%) cases of Hashimotos thyroiditis, 36 (7%) cases were with neoplasms other than papillary carcinoma, 30 (6%) cases were reported as cystic lesion of thyroid and (17) 4% cases as papillary carcinoma of thyroid*(figure 6).*

Figure 6: Distribution Of Disease



Of the 556 cases available, 86 cases were included in the study based on the inclusion and exclusion criteria.

Among the 86 cases, 13 cases were reported as papillary carcinoma thyroid in cytology and confirmed by follow up Histopathology report. For 17 cases, histopathology diagnosis of papillary carcinoma was given but cytology diagnosis was that of non-papillary carcinoma. Among the 17 cases, 5 were reported as suspicious for papillary carcinoma on cytology, 6 as follicular neoplasms and the remaining 6 cases were diagnosed as non-neoplastic lesions. The diagnoses of the remaining 56 cases were that of non-papillary carcinoma, which included neoplasms other than papillary carcinoma and non - neoplastic lesions.

Among the 30 cases with histopathology diagnosis of papillary carcinoma, 13 cases (43%) were reported as papillary carcinoma, 5 cases (17%) were reported as suspicious for papillary carcinoma, 6 cases (20%) were reported as follicular neoplasm and 6 cases (20%) were reported as non-neoplastic lesions in cytology.

Amongst the papillary carcinoma cases, the histopathology diagnosis were as follows; 20 cases were of the conventional papillary carcinoma type, 8 were of the follicular variant of papillary carcinoma and 2 were reported as oxyphilic variant of papillary carcinoma.

Of the 6 cases reported as suspicious for papillary carcinoma in cytology, 5 cases (83%) turned out to be conventional variant of papillary carcinoma and 1 case (17%) turned out to be multinodular goitre with adenomatoid change on follow-up histopathologic diagnosis.

Of the 17 cases reported as follicular neoplasm in the cytology, 6 (35%) cases turned out to be papillary carcinoma, among which 4 cases were reported as follicular variant of papillary carcinoma and 2 cases were reported as oncocytic variant of papillary carcinoma.

Among the remaining 50 cases reported as non-neoplastic lesions in cytology, 6 (12%) cases turned out to be papillary carcinoma, 3 cases (6%) were reported as follicular carcinoma, 9 cases (18%) were reported as follicular adenoma, 7 cases (14%) were reported as Hashimotos thyroiditis, 25 cases (50%) were reported as colloid goitre with associated changes in the following histopathology reports.

The nuclear features are summarized below.

NUCLEAR GROOVES:

Among the 30 cases of histologically proven papillary carcinoma cases, 17 (57%) cases had nuclear grooves ≥ 20% *(table 2),* 12 (40%) cases had nuclear grooves ranging between 10 and 19% *(table 3)* and 1 (3%) case with nuclear grooves < 10% *(table 4).*

Among the 20 cases of conventional papillary carcinoma type, 16 (80%) cases showed nuclear grooves to be ≥ 20%, 3 (15%) cases showed nuclear grooves to be between 10 and 19% and 1 (5%) case showed nuclear grooves to be < 10%. From the above details it can be inferred that all cases with ≥ 20% nuclear grooves in cytology, were histopathologically diagnosed as papillary carcinoma thyroid, except one case where the diagnosis was that of follicular carcinoma.

In the 8 cases of follicular variant of papillary carcinoma, no case showed ≥ 20% nuclear grooves. All the 8 (100%) cases showed nuclear grooves between 10 and 19%.

Both the oncocytic variants of papillary carcinoma showed nuclear grooves between 10 and 19%.

Among the remaining 56 cases of non-papillary carcinoma lesions, 1 (2%) case showed nuclear grooves ≥ 20%, 12 (21%) cases had nuclear grooves between 10 and 19% and 43 (77%) cases showed < 10% nuclear grooves.

The specificity and sensitivity of nuclear grooves for papillary carcinoma in the category of ≥ 20% were 98.2% and 50 % respectively and the same in the category of > 10% were 78.57% and 93.3% respectively.

Table 2: NUCLEAR GROOVES ≥20%

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
PAPILLARY CARCINOMA	12	0
SUSPICIOUS OF PAPILLARY CARCINOMA	4	0
FOLLICULAR NEOPLASM	0	1

Table 3: NUCLEAR GROOVES 10-19%

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
SUSPICIOUS OF PAPILLARY CARCINOMA	2	0
FOLLICULAR NEOPLASM	6	2
CYSTIC LESION THYROID	2	0
HASHIMOTOS THYROIDITIS	1	0
COLLOID GOITRE	2	10

Table 4: NUCLEAR GROOVES <10%

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
NODULAR COLLOID GOITRE	1	36
FOLLICULAR NEOPLASM	0	7

Figure 7: Distribution Of Cases based on percentage of Nuclear Grooves

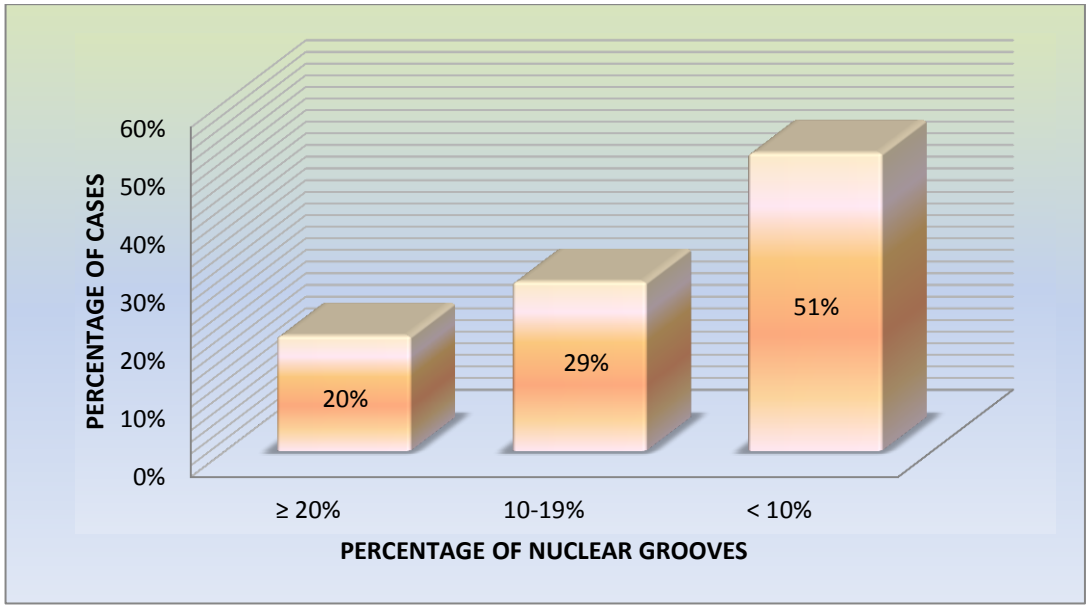
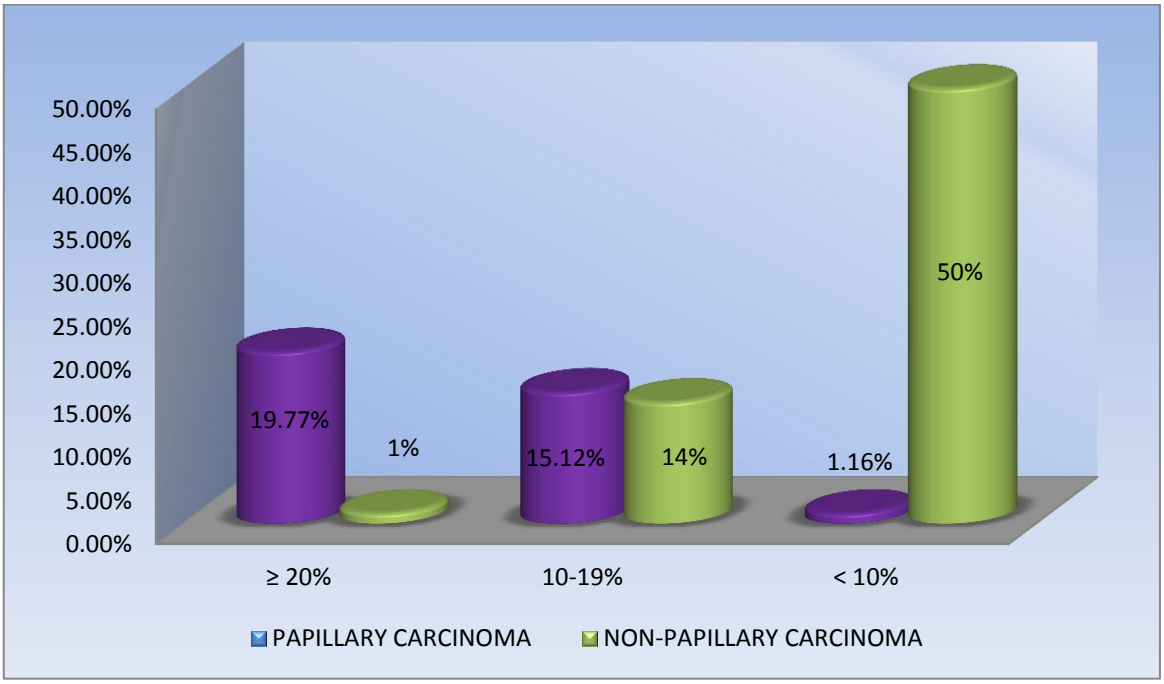


Figure 8: Distribution of Papillary Carcinoma and Non-Papillary Carcinoma cases based on percentage of Nuclear Grooves:



INTRANUCLEAR CYTOPLASMIC INCLUSIONS:

In the 30 cases of histologically proven papillary carcinoma, 11 (37%) cases showed frequent pseudoinclusions *(table 5)*, 12 (40%) cases showed infrequent pseudoinclusions and 7 (23%) cases showed absence of inclusions.

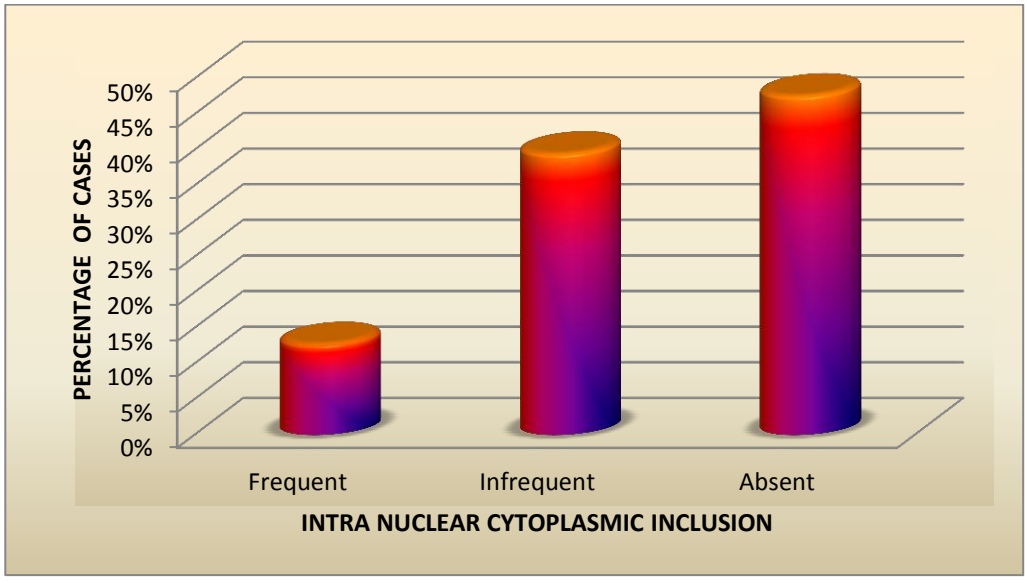
It is to be noted that pseudoinclusions were not frequently seen in any of the non-papillary carcinoma cases.

The specificity and sensitivity of pseudoinclusions for papillary carcinoma thyroid were 100% and 36.67% respectively.Their positive predictive value and negative predictive values were 100% and 74.67% respectively.

Table 5: FREQUENT PSEUDOINCLUSIONS

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
PAPILLARY CARCINOMA	9	0
FOLLICULAR NEOPLASM	1	0
ADENOMATOUS NODULE	1	0

Figure 9: Distribution of Cases based on Frequency of Pseudo-Inclusion



PAPILLARY FRAGMENTS:

Among the 30 cases of histologically confirmed papillary carcinoma, papillary fragments were seen in 16 (53%) *(table6)* cases and were absent in the remaining cases.

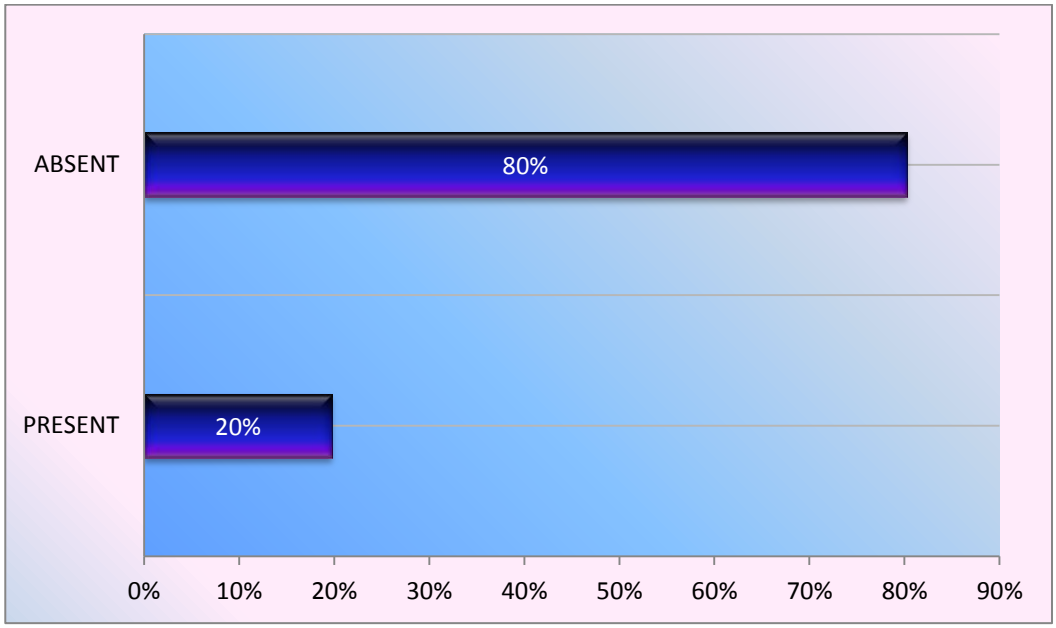
Only one case other than papillary carcinoma showed papillary fragments which was diagnosed as follicular adenoma with papillary hyperplasia in the following excision biopsy sent for histopathology.

The specificity and sensitivity of papillary fragments for papillary carcinoma thyroid were 98.21 % and 53.33 % respectively.Their positive predictive value and negative predictive values were 94.12% and 79.71% respectively.

Table 6: PAPILLARY FRAGMENTS

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
PAPILLARY CARCINOMA	8	0
SUSPICIOUS OF PAPILLARY CARCINOMA	4	0
FOLLICULAR NEOPLASM	3	1
ADENOMATOUS NODULE	1	0

Figure 10: Distribution of Cases based on Presence of Papillary Fragments



METAPLASTIC CYTOPLASM :

Among the 30 cases of histologically proven papillary carcinoma, metaplastic cytoplasm was seen in 21*(table 7)* (70%) cases and this feature was absent in the remaining cases.

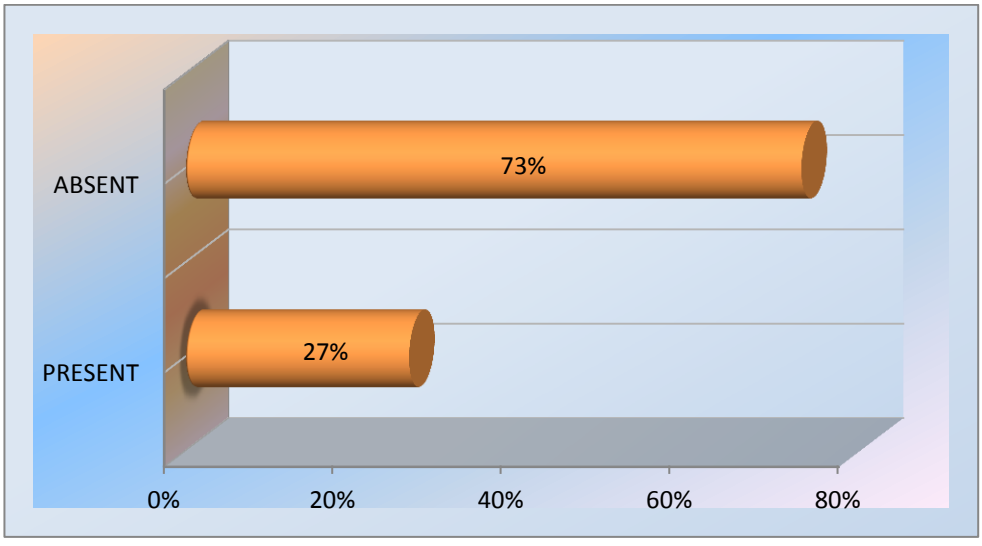
Two other cases also showed metaplastic cytoplasm which histologically had the diagnosis of follicular carcinoma.

The specificity and sensitivity of metaplastic cytoplasm for papillary carcinoma thyroid were 96.43% and 70 % respectively.Their positive predictive value and negative predictive values were 91.30% and 85.71% respectively.

Table 7: METAPLASTIC CYTOPLASM

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
PAPILLARY CARCINOMA	9	0
SUSPICIOUS OF PAPILLARY CARCINOMA	5	0
FOLLICULAR NEOPLASM	4	1
COLLOID GOITRE	3	1

Figure 11: Distribution of Cases based on presence of Metaplastic Cytoplasm



THREE DIMENSIONAL FRAGMENTS (TDF):

Among the 30 cases of histologically proven papillary carcinoma, TDF were seen in 22 *(table 8)* (73%) cases and were absent in the remaining cases.

15 cases with a diagnosis other than papillary carcinoma also showed TDF, amongst which 9 cases were reported as colloid goitre and 3 cases were reported as adenomatous nodule, 4 cases were reported as follicular adenoma and 2 cases were reported as follicular carcinoma in the follow up histopathology reports.

The specificity and sensitivity of Three Dimensional fragments for papillary carcinoma thyroid were 73.21 % and 73.33 % respectively.

The positive predictive value (probability that the disease is present when the test is positive) and negative predictive value (probability that the disease is not present when the test is negative) of three dimensional fragments for papillary thyroid carcinoma were 59.46% and 83.67% respectively.

Table 8: Three Dimensional Fragments [TDF]

CYTOLOGY DIAGNOSIS	HISTOPATHOLOGY REPORT	
	PAPILLARY CARCINOMA	NON-PAPILLARY CARCINOMA
PAPILLARY CARCINOMA	10	0
SUSPICIOUS OF PAPILLARY CARCINOMA	4	0
FOLLICULAR NEOPLASM	5	3
HASHIMOTOS THYROIDITIS	1	0
ADENOMATOUS NODULE	2	1
COLLOID GOITRE	0	6

Figure 12: Distribution of Cases based on presence of TDF

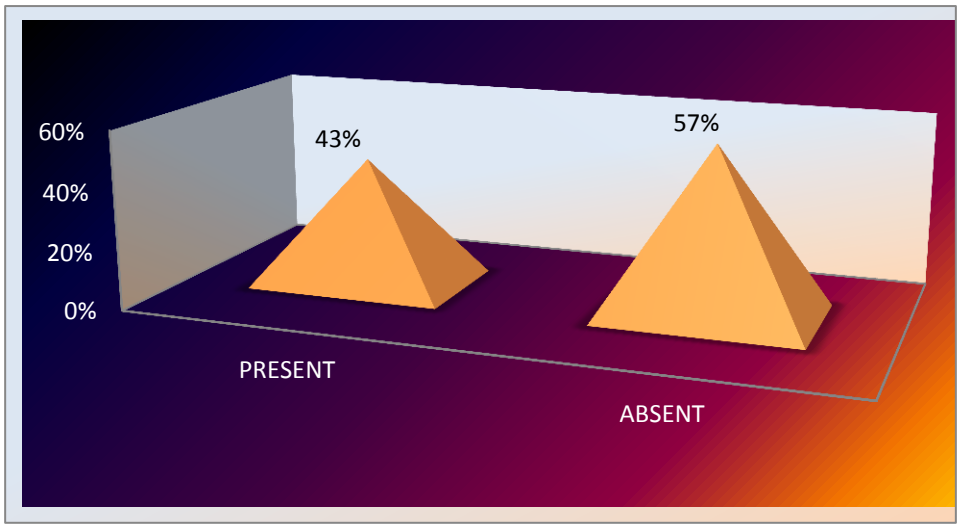
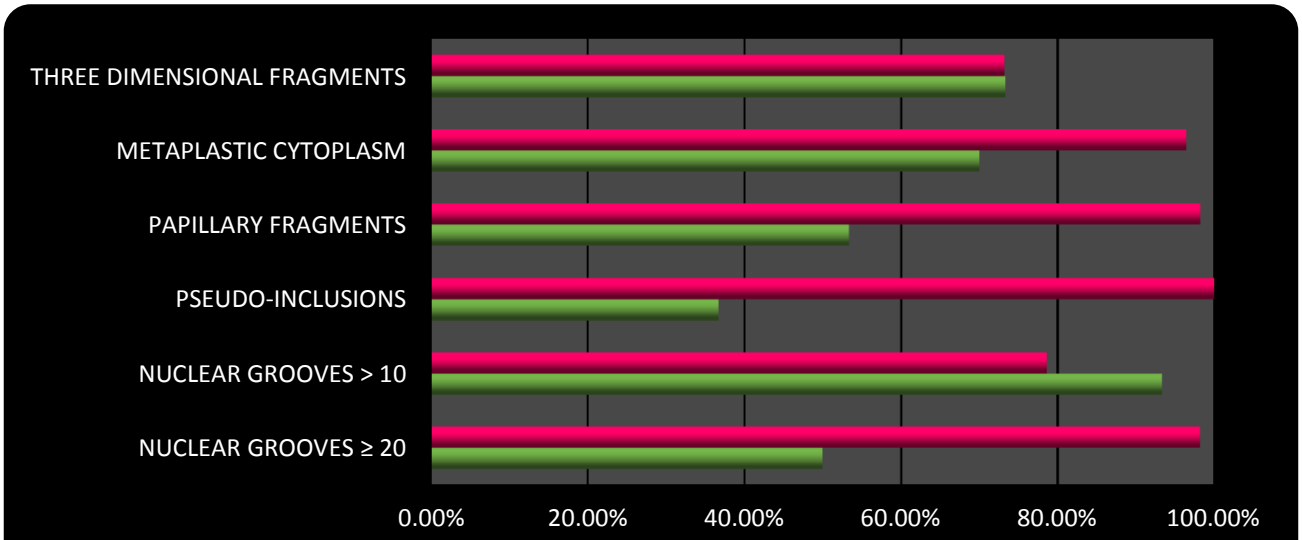


Figure 12: Sensitivity and Specificity of all the 5 Nuclear features for Papillary Carcinoma



	NUCLEAR GROOVES ≥ 20	NUCLEAR GROOVES > 10	PSEUDO-INCLUSIONS	PAPILLARY FRAGMENTS	METAPLASTIC CYTOPLASM	THREE DIMENSIONAL FRAGMENTS
Specificity	98.20%	78.57%	100%	98.21%	96.43%	73.21%
Sensitivity	50.00%	93.30%	36.67%	53.33%	70%	73.33%

DISCUSSION

Papillary carcinoma thyroid is the most common type of thyroid cancer. Prognosis of these patients is good with 5 year survival rate of 96% and 10 year survival rate of 93%[70]. The accuracy of report is very high with fine needle aspiration cytology and hence, Fine Needle Aspiration is indicated for all cases presenting with a thyroid swelling.

Among the various features of papillary carcinoma thyroid known, presence of nuclear grooves is still recognized as a much necessary criteria in the diagnosis of papillary carcinoma ever since its identification in 1986[71].

Many studies conducted earlier have concluded that, to arrive at a diagnosis of papillary carcinoma, combinations of cyto-morphological features are necessary. Some of such studies are mentioned below.

A study conducted by Kini et al. and colleagues in 1980, defined six cytologic features for describing papillary carcinoma of thyroid including monolayered sheets of cells, intra-nuclear cytoplasmic inclusions, papillary structure, presence of follicular pattern, foreign body type multi- nucleated giant cell and absence of degenerative changes[72]. Later in 2003, a study conducted by Castro-Gomez et al. included fifteen features to define papillary carcinoma[59].

Later Wu et al, came up with an idea of including seven of the most commonly occurring cytomorphologic features for papillary carcinoma namely: cells arranged in syncytial sheets, enlargement of the nucleus, nucleus with fine chromatin distribution, presence of nuclear grooves, intra nuclear cytoplasmic inclusions, presence of scant colloid and little cytoplasm[73].

Inspite of having many well described features to diagnose papillary carcinoma, difficulty in diagnosis doexist even now, in making a diagnosis of papillary carcinoma. Even in our study, only 13 among the 30 histologically proven papillary carcinoma cases were diagnosed in cytology as papillary carcinoma.

In 2009, Dilip K. Das and his colleague Prem N. Sharma conducted a study to evaluate the constraint in making a decision of papillary carcinoma of thyroid in aspiration smears. They included five cyto–morphological features such as nuclear grooves, intranuclear cytoplasmic inclusions, papillary fragments, fine chromatin pattern and psammoma bodies. In their conclusion, they have mentioned that the diagnosis of papillary carcinoma thyroid becomes easier if ≥ 3 out of 5 above mentioned cytologic features are present. They have also mentioned that in circumstances where the number of cytologic features are stingent and are less than 3 then the most dependable nuclear features in favour of papillary carcinoma will be nuclear grooves and intra nuclear cytoplasmic inclusions[67]

In routine day to day practice, making a diagnosis on thyroid FNA is becoming increasingly difficult and frustrating due to the lack of certainty with regards to the significant presence of nuclear grooves. This becomes even more difficult when there is lack of other diagnostic features of papillary carcinoma other than nuclear grooves, as nuclear grooves can also be present in other non-papillary carcinoma thyroid lesions.

In our study we included five nuclear features namely nuclear grooves, intranuclear cytoplasmic inclusions, presence of papilla, presence of metaplastic cytoplasm and presence of three dimensional fragments to analyse the individual occurrence of these features in papillary carcinoma cases and other thyroid lesions.

As with many other previous studies, our study also confirms that, presence of both nuclear grooves and intranuclear cytoplasmic inclusions are diagnostic of papillary carcinoma. The significance of these features were not extensively analysed in previous studies. We have tried to look in to these features individually and derive a cut-off value to distinguish between papillary carcinoma and other non-papillary carcinoma lesions.

The quantitative approach for nuclear grooves is being debated over three decades since its discovery in 1986.

In 1989, Rupp et al. compared the number of nuclear grooves present in papillary carcinoma and in benign lesions by evaluating percentage of nuclear grooves in 30 HPF. He concluded that, occurrence of nuclear grooves in benign lesions are very less compared to their occurrence in papillary carcinoma[74]. Gould et al. also performed a similar kind of study in the same year but he evaluated the percentage of nuclear grooves in 5 HPF and he came up with a similar conclusion that the occurrence of nuclear grooves is more frequent in papillary carcinoma in comparison to its presence in other benign thyroid lesions[75]. In 1995, Fransis et al did a similar study but he adopted a new quantitative method of evaluating percentage of nuclear grooves in 500 cells rather than counting cells in different fields as in the previous studies. His results were also the same as others.

In 2003, Yi Jun Yang conducted a study by adopting the semi-quantitative method of Gould et al. and evaluated percentage of nuclear grooves in 5 HPF where it occurred commonly and came up with a diagnostic specificity and sensitivity for papillary carcinoma. According to their study, the sensitivity of cells with > 10% nuclear grooves was 100% and the sensitivity reduced to 65% at the level of ≥ 20% grooves[62].

In our study we followed a quantitative method by counting 500 cells and then calculated the percentage of nuclear grooves. We then derived the sensitivity

and specificity of nuclear grooves in diagnosing papillary carcinoma as given in *table 2.*

According to our data, the diagnostic sensitivity is 93.3% at the level of >10% grooves but the sensitivity drops to 50% when only cells with ≥ 20% grooves are considered. Hence, it is observed from the above data that, sensitivity is high when the criteria is kept as low as > 10% nuclear grooves and the sensitivity reduces when the criteria of ≥ 20% are considered. It is a known fact that nuclear grooves are a characteristic feature of papillary carcinoma and their sensitivity is not to be debated. The main dispute is the specificity of nuclear grooves in diagnosing papillary carcinoma and also the numbers required to arrive at a diagnosis of papillary carcinoma.

According to a study conducted by Yang and Demirci, the specificity for diagnosis of papillary carcinoma is 95% at the level of ≥ 20% grooves and the specificity increases to 100% when all other neoplasms were also considered[62]. According to our data, the diagnostic specificity for papillary carcinoma at the level of ≥ 20% nuclear grooves is 98.2%. Only one case with ≥ 20% of nuclear grooves turned out to be follicular carcinoma. Hence, the diagnostic specificity is 100% for malignant neoplasm at the level of nuclear grooves ≥ 20%.

The above data clearly defines that, presence of ≥ 20% grooves is characteristically diagnostic of papillary carcinoma of thyroid.

Also our data states that the negative predictive value for papillary carcinoma with nuclear grooves < 10% is 97.7% from which we infer that, when nuclear grooves constitute only < 10% of the cell population, the probability of papillary carcinoma can be excluded.

At this point, it is to be noted that thyroid lesions other than papillary carcinoma can also show nuclear grooves but their frequency of occurrence will be very less. So the pathologists should make it a point to avoid over diagnosis of papillary carcinoma due to the mere presence of few nuclear grooves and thereby preventing its false positive diagnosis.

The difficulty in diagnosis arises when the nuclear grooves are between 10% and 20%. In our study the specificity for papillary carcinoma decreases to 78.57% at the level of 10 – 20%. According to a study conducted by Yang and Demirci, the diagnostic specificity at the level of 10 to 20% nuclear grooves fall to 68%. They concluded that in the absence of other contributory nuclear features such as intra nuclear cytoplasmic inclusions, papillary fragments, metaplatic cytoplasm and three dimensional fragments, a diagnosis of "suspicious for malignancy" or "atypical cytology" should be given to reduce the chance of false negativity[62]. In concordance with their study, our data also shows fall in specificity at this level and we concluded that a similar diagnosis

of “suspicious for malignancy” can be given to the cases which fall in this category of 10 to 19% grooves. In such “suspicious cases”, we should search carefully for intra nuclear cytoplasmic inclusions in addition to nuclear grooves to make a definitive diagnosis of papillary carcinoma. The presence of frequent intra nuclear cytoplasmic inclusions strongly indicates the diagnosis of papillary carcinoma.

In consistence with many previous studies, our study also showed fewer amount of nuclear grooves in the benign lesions. As a matter of fact, question arises regarding their specificity in the diagnosis of papillary carcinoma. Many authors say nuclear grooves are not specific in diagnosing papillary carcinoma of all the organs, but in thyroid, nuclear grooves have high specificity in diagnosing papillary carcinoma. It is to be noted that high percentage of nuclear grooves can also be observed in thyroid lesions other than papillary carcinoma. Hence, it is understood here that presence of other features such as intra nuclear cytoplasmic inclusions, papillary fragments, metaplastic cytoplasm and three dimensional fragments becomes more important in making a diagnosis or ruling out the probability of papillary carcinoma.

In most of the studies conducted previously, the nucleus was looked upon for intra nuclear cytoplasmic inclusions and mere presences of inclusions were labelled as “present pseudoinclusions” and were considered for analysis.

We followed a semi-quantitative method to find out the frequency of occurrence of intra nuclear cytoplasmic inclusions, which has been rarely reported in literature. Five random HPF were selected and looked for pseudoinclusions. When ≥ 5% cells showed pseudoinclusions they were labelled as frequent but when < 5% cells showed this feature they were labelled as infrequent and when none of the cells had pseudoinclusion, they were labelled absent.

A study conducted by Yi Jun Yang in 2003, found that frequent intra nuclear cytoplasmic inclusions had a diagnostic specificity of 100% for papillary carcinoma but the negative predictive value for intra nuclear cytoplasmic inclusions were only 61% as they can also be infrequently present in other lesions of thyroid. In consistence with this study[62], our data also shows that intra nuclear cytoplasmic inclusion has a diagnostic specificity of 100% but the negative predictive value was 74.67%. From the above data it can be inferred that, presence of intra nuclear cytoplasmic inclusions alone cannot be used as a diagnostic criteria for papillary carcinoma as the false negative rate is very high.

As with other studies, our data also illustrates that definitive diagnosis of papillary carcinoma thyroid is difficult when only nuclear grooves are encountered in the absence of pseudoinclusions.

In our study, the variant of papillary carcinoma which was missed more frequently in cytology was the follicular variant of papillary carcinoma. According to a study conducted by Perez et al in 2001,the frequency of nuclear grooves and intra nuclear cytoplasmic inclusions are much less in follicular variant of papillary carcinoma as compared to that of the conventional variant. Yi Jun Yang, in his study conducted during the year 2003 gave a similar conclusion[62]. In concordance with both their studies, we also had animpression that follicular variant is being missed in cytology due to their paucity of features of conventional papillary carcinoma.

In most of the studies, papillary fragments were considered along with other features in the diagnosis of papillary carcinoma. In our study, we evaluated Positive predictive value (i.e.) probability that the disease is present when the papillary fragments are present to be 94.12% and negative predictive value (i.e.) probability that the disease is not present when papillary fragment is absent is 79.71%. This tells us that a diagnosis of papillary carcinoma cannot be considered when only papillary fragments are seen as the possibility of false negative rate will be unacceptably high.

From our study, we conclude that papillary fragments along with other specific features like nuclear grooves and intra nuclear cytoplasmic inclusion can be helpful to arrive at a diagnosis of papillary carcinoma.

In most of the studies, metaplastic cytoplasm was not studied as an individual feature but was considered along with other specific features of papillary carcinoma for its diagnosis. In our study, we analysed this feature individually and derived the following values with the available data. According to our data, Positive predictive value (i.e.) probability that the disease is present when the metaplastic cytoplasm is present is 91.30% and negative predictive value (i.e.) probability that the disease is not present when metaplastic cytoplasm is absent is 85.71%.

Our conclusion with regard to metaplastic cytoplasm is that, along with other specific features like nuclear grooves and intra nuclear cytoplasmic inclusion, metaplastic cytoplasm can act as a helpful tool to arrive at a diagnosis of papillary carcinoma.

Similar to the above two features, we considered three dimensional fragments individually, and derived the following values with the available data. According to our data, Positive predictive value (i.e.) probability that the disease is present when the three dimensional fragments are present is 59.46%and negative predictive value (i.e.) probability that the disease is not present when three dimensional fragments are absent is 83.67%. In other words, if one suspects a diagnosis of papillary carcinoma by considering the

presence of three dimensional fragments alone, the false positive rates will be unacceptably high.

Based on the above said data, we inferred that three dimensional fragments alone cannot serve to make a specific diagnosis of papillary carcinoma but, this in combination with other specific features such as nuclear grooves, intra nuclear cytoplasmic inclusions, papillary fragments and metaplastic cytoplasm can act as an important feature in the diagnosis of papillary carcinoma.

According to the results obtained from our data, we observed that atleast two of the above mentioned features were present in addition to nuclear grooves in all cases of papillary carcinoma. Hence, a diagnosis of papillary carcinoma can be made when frequent nuclear grooves are present along with atleast two other features mentioned in our study.

SUMMARY AND CONCLUSION

- The five morphological features studied and analysed on FNA of papillary and non papillary carcinoma cases were nuclear grooves, intranuclear cytoplasmic inclusions, papillary fragments, metaplastic cytoplasm and three dimensional fragments.
- The feature that occurred most commonly in papillary as well as non papillary carcinoma cases was nuclear grooves.
- ≥ 20% grooves were found to be diagnostic of papillary carcinoma with specificity of 98.20%
- The feature most specific for papillary carcinoma was frequent Intra Nuclear Cytoplasmic Inclusion (≥ 5%)
- However, ≥ 20% nuclear grooves and frequent intranuclear inclusions in combination favoured diagnosis of papillary carcinoma in most of the cases
- Papillary fragments, metaplastic cytoplasm and presence of three dimensional fragments were not found to be individually specific for the diagnosis of papillary carcinoma but, any of the two above mentioned features in addition to frequent nuclear grooves favoured the diagnosis of papillary carcinoma.
- In addition, presences of < 10% nuclear grooves favoured the diagnosis of benign thyroid lesion and clearly ruled out malignancy.

- The cases that fall in the category of “suspicious of malignancy” (i.e.) those with nuclear grooves between 10 and 19% have to be dealt with caution. In such cases, the combination of other features plays an important role in correct diagnosis of papillary carcinoma and is a better indicator of the appropriate treatment protocol.
- Follicular variant of papillary carcinoma is the common variant which is missed in cytology due to the paucity of nuclear features.
- In view of the above mentioned points, the pathologists should be aware that the cytomorphological features of papillary carcinoma can also be seen in other thyroid lesions and, should be cautious not to over diagnose papillary carcinoma.
- A larger study including more morphological features can go a long way in accurately diagnosing papillary carcinoma thyroid.

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No.	CYTOLOGY NO. & DIAGNOSIS	HP NO. & DIAGNOSIS	CYTOLOGY FINDINGS				
			nuclear grooves [%]	pseudo inclusions	papillary	metaplastic cytoplasm	TDF
1	159/10- Nodular Colloid Goitre	389/10- Follicular Adenoma	2	Absent	Absent	Absent	Absent
2	195/10- F/S/O Hyperplastic Nodular Goitre	1100/10- Nodular Colloid Goitre	2	Absent	Absent	Absent	Present
3	605/10- A] Adenomatoid Nodule B]Follicular Adenoma	1110/10- Follicular Adenoma	5	Infrequent	Absent	Absent	Present
4	3065/09-F/S/O Nodular hyperplasia with toxic change	1114/10- Follicular Adenoma	5	Absent	Absent	Absent	Present
5	1372/10- Nodular colloid Goitre	2322/10- Nodular Goitre with cystic change	4	Absent	Absent	Absent	Absent
6	687/10- A] Follicular neoplasm B]Adenomatous change in NCG	2644/10- Colloid Nodule	7	Absent	Absent	Absent	Present
7	2204/10- NCG with cystic change	3766/10- Follicular Adenoma	6	Infrequent	Absent	Absent	Absent
8	2040/10- A] Follicular neoplasm B]Adenomatous change in nodular colloid goitre	3958/10- Follicular Adenoma	8	Infrequent	Absent	Absent	Absent
9	2511/10- Follicular Neoplasm to R/O Papillary carcinoma	4087/10- MNG with adenomatoid change	12	Infrequent	Absent	Absent	Present
10	2816/10- A] Follicular neoplasm B]Follicular varient of papillary carcinoma	4407/10- Follicular carcinoma	22	Infrequent	Absent	Absent	Present

11	2815/10 A] Adenomatous change in nodular goitre B]Follicular neoplasm	4468/10- NCG with adenomatous change	11	Infrequent	Absent	Absent	Present
12	225/11 NCG with cystic change	552/11 - nodular hyperplasia	2	Absent	Absent	Absent	Absent
13	576/11- NCG	817/11- NCG	2	Absent	Absent	Absent	Absent
14	681/11- NCG	910/11- Nodular Hashimotos Thyroiditis	6	Infrequent	Absent	Absent	Absent
15	812/11- NCG	999/11- Nodular Hyperplasia	5	Absent	Absent	Absent	Absent
16	951/11- F/s/o Adenomatous change in a NCG	1211/11- NCG	15	Infrequent	Absent	Absent	Present
17	1074/11- NCG with cystic change	1482/11- NCG	2	Infrequent	Absent	Absent	Absent
18	961/11- NCG	1743/11 - Adenomatous Nodule	7	Infrequent	Absent	Absent	Absent
19	875/11- Colloid Goitre	1975/11- NCG with Adenomatous nodule	5	Absent	Absent	Absent	Present
20	1455/11- F/S/O NCG	2463/11- Nodular Hyperplasia	3	Absent	Absent	Absent	Present
21	41/12- NCG	230/12- NCG	3	Absent	Absent	Absent	Absent
22	1884/12- Adenomatous change in NCG	2015/12- NCG	1	Infrequent	Absent	Absent	Absent
23	1957/12- NCG with cystic change	2082/12- NCG with focal Lymphocytic Thyroiditis	6	Absent	Absent	Absent	Absent
24	982/12- Colloid Goitre	2110/12- colloid goitre	2	Absent	Absent	Absent	Absent
25	1535/12- NCG with cystic change	2228/11- Dyshormonogenic Goitre	8	Absent	Absent	Absent	Absent
26	2321/12- Nodular Goitre with cystic change	2764/12- nodular goitre	9	Infrequent	Absent	Absent	Absent
27	1647/12- F/O NCG	3014/12- Nodular Hyperplasia	14	Infrequent	Absent	Absent	Present

28	2359/12- Nodular Goitre with Cystic change	2841/12- Nodular Hashimoto Thyroiditis	7	Infrequent	Absent	Absent	Absent
29	2567/12- NCG with Cystic change	3092/12- MNG with Retrogressive changes	5	Infrequent	Absent	Absent	Absent
30	2657/12- Adenomatous Change in NCG	3209/12- Follicular carcinoma(Rt lobe),Adenomatous Hyperplasia(Lt lobe)	17	Infrequent	Absent	Absent	Present
31	222/12- Nodular Hyperplasia with cystic degeneration	3375/12- Nodular Hyperplasia with Retrogessive changes	18	Infrequent	Absent	Absent	Absent
32	2803/12- F/S/O cystic change in NCG	3399/12- Nodular Goitre	6	Infrequent	Absent	Absent	Absent
33	2670/12- S/O Follicular Neoplasm	3423/12- Nodular Goitre	3	Absent	Absent	Absent	Absent
34	2679/12- NCG	3477/12- Nodular Toxic Hashimotos Thyroiditis	7	Absent	Absent	Absent	Absent
35	3107/12- Nodular Goitr with cystic change	3787/12- Nodular Goitre	5	Absent	Absent	Absent	Absent
36	2823/12- NCG with cystic change	4114/12- Multinodular Dyshormonogenic Goitre	6	Infrequent	Absent	Absent	Absent
37	3473/12- Colloid Goitre with cystic change	4316/12- Nodular Hyperplasia	4	Absent	Absent	Absent	Absent
38	3496/12- Follicular Neoplasm	4349/12- Follicular Adenoma with focal Papillary Hyperplasia	9	Infrequent	Absent	Absent	Present
39	3616/12- NCG	4663/12- Nodular Goitre with Hashimotos Thyroiditis	2	Absent	Absent	Absent	Absent
40	3811/12- S/O NCG	4960/12 - Nodular Goitre	8	Infrequent	Absent	Absent	Absent
41	follicular neoplasm	follicular carcinoma	17	Infrequent	Absent	present	Absent
42	adenomatous change in nodular goitre	NCG with adenomatous change	13	Infrequent	Absent	Absent	Absent

43	adenomatous change in nodular goitre	NCG	14	Infrequent	Absent	Absent	Absent
44	nodular colloid goitre	nodular hyperplasia	16	Infrequent	Absent	Absent	Absent
45	adenomatous change in nodular goitre	follicular carcinoma	19	Infrequent	Absent	present	Absent
46	nodular hyperplasia	nodular hyperplasia	18	Infrequent	Absent	Absent	Absent
47	nodular colloid goitre	follicular adeoma	2	Absent	Absent	Absent	Absent
48	follicular adenoma	follicular adenoma	5	Infrequent	Absent	Absent	Present
49	follicular neoplasm	colloid nodule	7	Absent	Absent	Absent	Absent
50	NCG	Nodular hashimotos thyroiditis	6	Infrequent	Absent	Absent	Absent
51	colloid goitre	NCG with adenomatous change	6	Infrequent	Absent	Absent	Present
52`	NCG with cystic change	NCG with focal lymphocytic thyroiditis	4	Absent	Absent	Absent	Absent
53	nodular goitre with cystic change	nodular goitre	8	Infrequent	Absent	Absent	Absent
54	NCG	toxic hashimotos thyroiditis	6	Infrequent	Absent	Absent	Absent
55	NCG with cystic change	multinodular dyshormonogenic goitre	7	Infrequent	Absent	Absent	Absent
56	follicular neoplasm	follicular adenoma with papillary hyperlasia	4	Infrequent	present	Absent	Absent
57	465/10- cystic papillary ca	1115/10- Papillary carcinoma thyroid	27	frequent	Absent	Present	Present
58	1151/10- F/O Papillary Carcinoma	2265/10- Cystic papillary Carcinoma	31	frequent	Present	Present	Present
59	1541/10- Papillary Carcinoma	2637/10- Papillary Carcinoma	22	frequent	Present	Present	Present
60	1902/10- Follicular Neoplasm, Possibility Of Papillary Carcinom	3149/10- Papillary carcinoma	20	frequent	Absent	Absent	Present

61	2320/10- suspicious of papillay carcinoma	3725/10- Follicular variant of papillary carcinoma	18	infrequent	Absent	Present	Present
62	2381/10- Follicular Neoplasm	4092/10- Follicular Variant Of Papillary Carcinoma	14	infrequent	Absent	Present	Present
63	803/11- Cystic Lesion Thyroid	1065/11- Papillary Carcinoma	11	infrequent	Absent	Absent	Absent
64	980/11- F/S/O Papillary Carcinoma	1259/11- Papillary Carcinoma	25	frequent	Present	Present	Present
65	3241/12- S/O Papillary Carcinoma	4006/12- Papillary Carcinoma	35	frequent	Present	Present	Present
66	3591/12- F/S/O papillary Carcinoma	4500/12- Papillary Carcinoma	27	infrequent	Absent	Absent	Present
67	503/13 - F/S/O papillary carcinoma	907/13- solid variant of papillary carcinoma	32	frequent	present	present	present
68	1246/13- cystic thyroid lesion .S/O papillary ca	1946/13- Follicular variant of papillary ca	12	absent	Present	Absent	Absent
69	2994/12- Follicular Neoplasm	1739/13- Follicular variant of Papillary ca	18	infrequent	Absent	Absent	Present
70	1485/13- follicular neoplasm	2184/13- oxiphilic varient of papillary ca	16	infrequent	Present	Present	Absent
71	2028/13- F/O Adenomatous Nodule	4226/13- Multifocal papillary ca	22	frequent	Absent	Present	Present
72	978/13- adenomatous nodule with hashimoto	4666/13- Multifocal papillary ca	14	absent	absent	absent	Present
73	2344/14- Adenomatous change in NCG	3427/14- Follicular variant of papillary ca	12	absent	Absent	Present	Absent
74	1840/14- papillary carcinoma	2732/14- papillary carcinoma	32	frequent	present	present	present
75	4342/13- colloid goitre with cystic change	356/14- papillary carcinoma	8	absent	Absent	Present	Present
76	4501/13- S/O papillary carcinoma	7/14- papillary carcinoma	27	infrequent	present	Present	Absent
77	suspicious of papillary carcinoma	follicular varient of papillary carcinoma	15	absent	Absent	Present	Present
78	follicular neoplasm	follicular varient of papillary carcinoma	15	infrequent	Present	Present	Present

79	follicular neoplasm	follicular varient of papillary carcinoma	17	infrequent	Absent	Absent	Present
80	follicular neoplasm	oxyphilic varient of papillary carcinoma	15	absent	Present	Present	Absent
81	adenomatous nodule	multifocal papillary carcinoma	16	infrequent	Present	Absent	Present
82	F/S/O Papillary carcinoma	Papillary carcinoma	27	infrequent	Present	Present	Present
83	S/O papillay carcinoma	Papillary carcinoma	30	frequent	Present	Present	Present
84	F/O Adenomatous nodule	multifocal papillary carcinoma	24	infrequent	Absent	Absent	Absent
85	Papillary carcinoma	Papillary carcinoma	34	frequent	Present	Present	Present
86	Papillary carcinoma	Papillary carcinoma	23	absent	Present	Present	absent

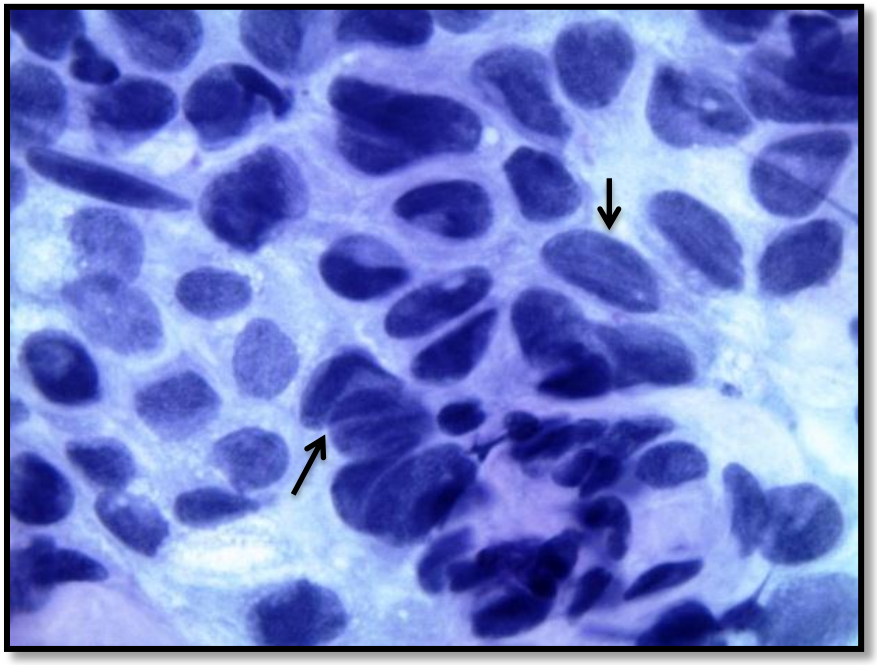

Figure 13: Nuclear Grooves(arrow) in papillary carcinoma. Papanicolaou stain; 100X

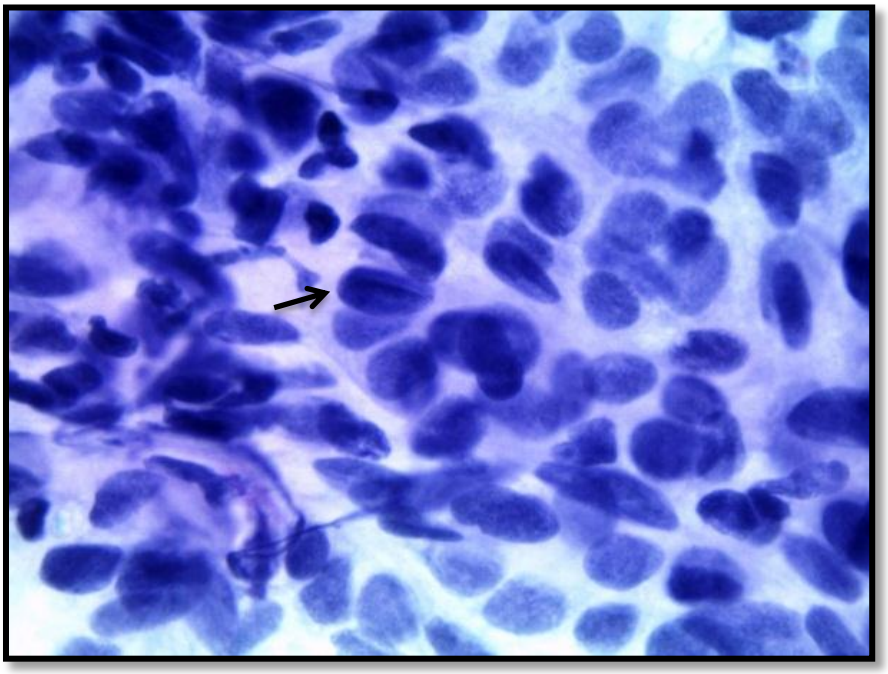

Figure 14: Nuclear Grooves in papillary carcinoma. Papanicolaou stain; 40X

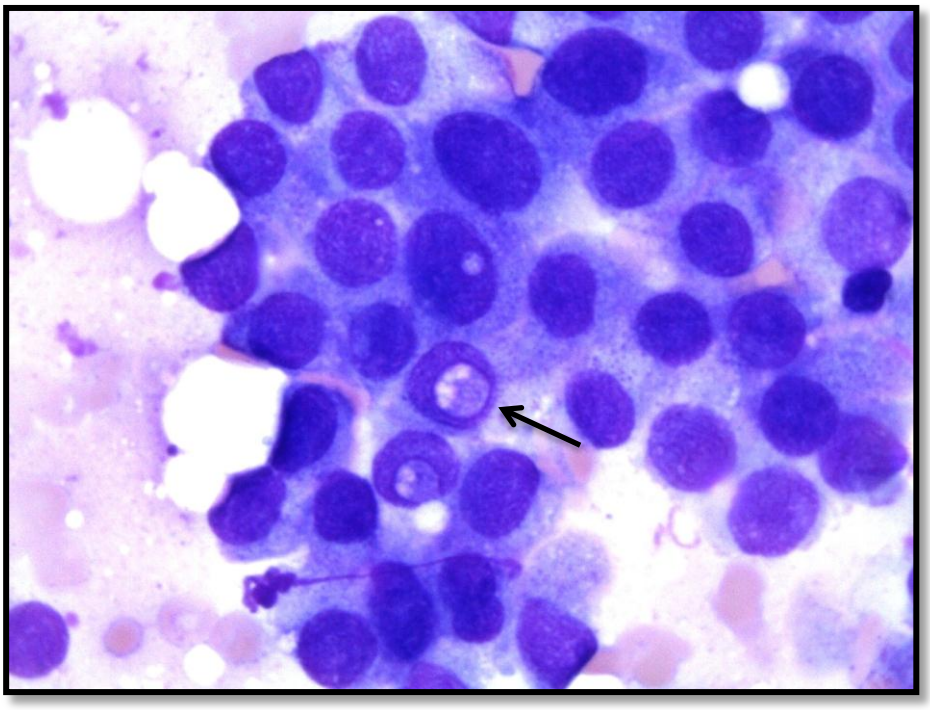

Figure 15: Intra-nuclear cytoplasmic inclusion (arrow) in papillary carcinoma. Papanicolaou stain; 100X

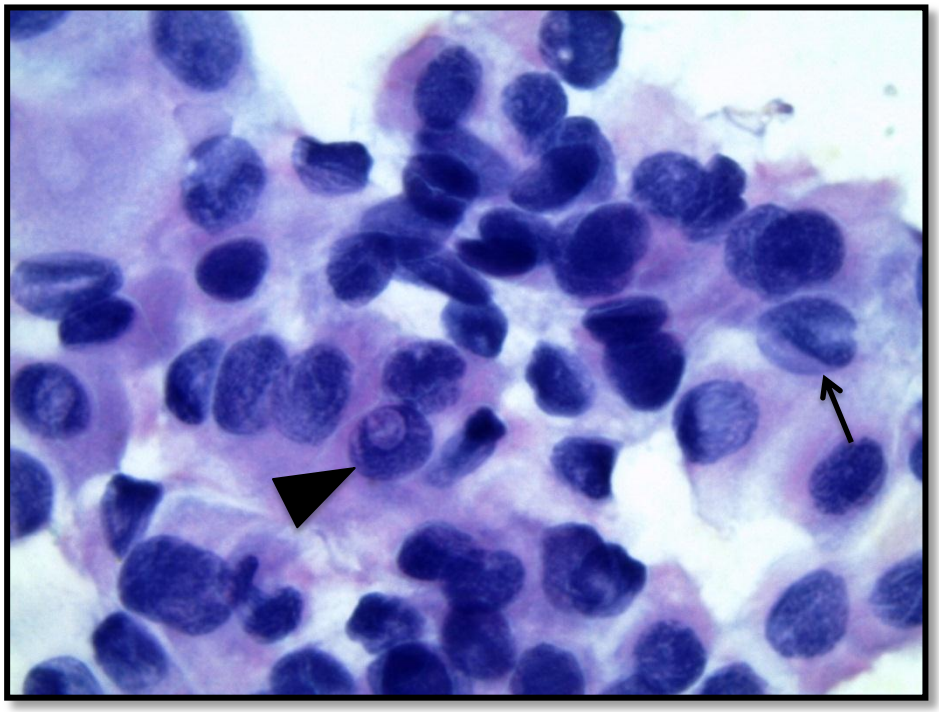

Figure 16: Nuclear Grooves (Arrow) and Intranuclear cytoplasmic inclusion (Arrowhead) in papillary carcinoma. Giemsa stain; 100X

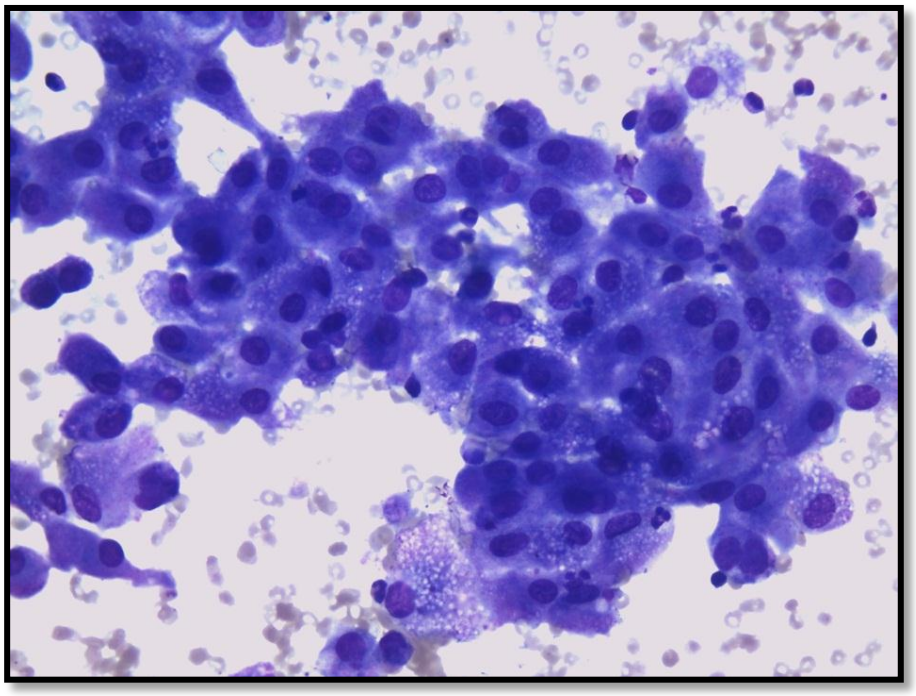

Figure 17: Metaplastic cytoplasm in Papillary Carcinoma. Giemsa stain; 40X

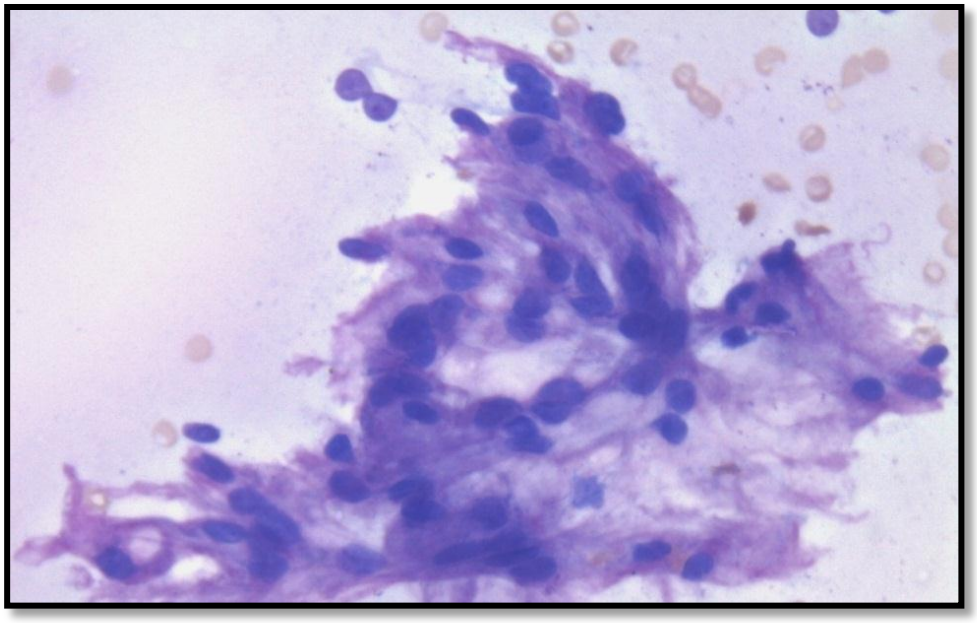

Figure 18: Metaplastic cytoplasm in Papillary Carcinoma. Giemsa stain; 40X

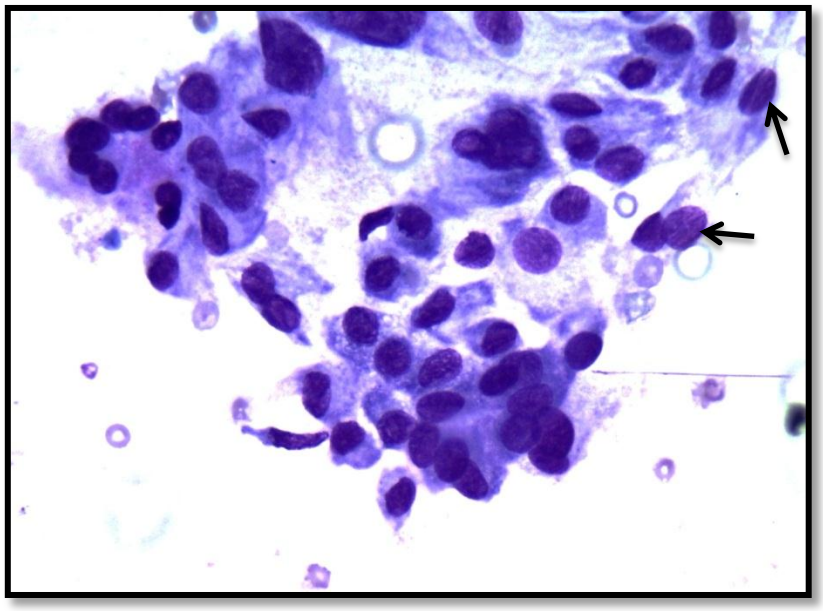

Figure 19: Nuclear Grooves (Arrows) In Adenomatous change in nodular goitre.

Papanicolaou stain; 40X

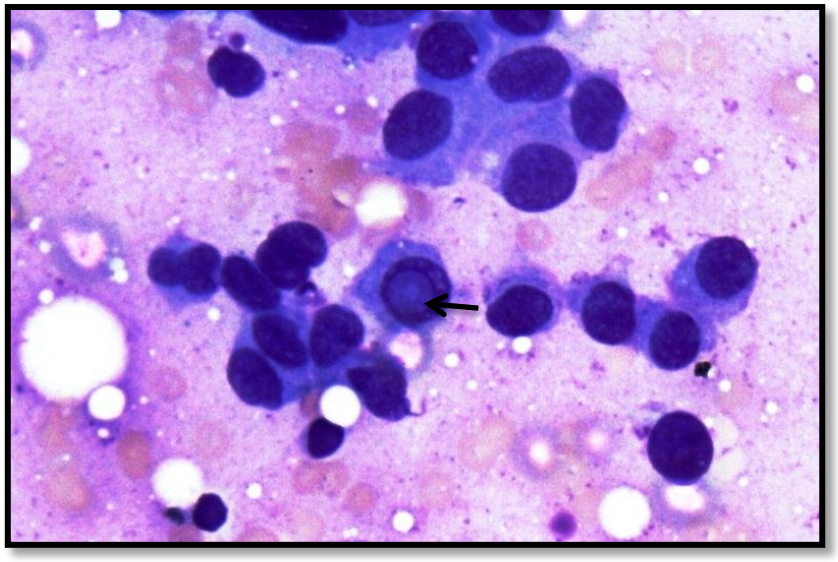

Figure 20: Intranulcear Cytoplasmic inclusion in a case of Hashimoto thyroiditis.

Giemsa stain; 40X

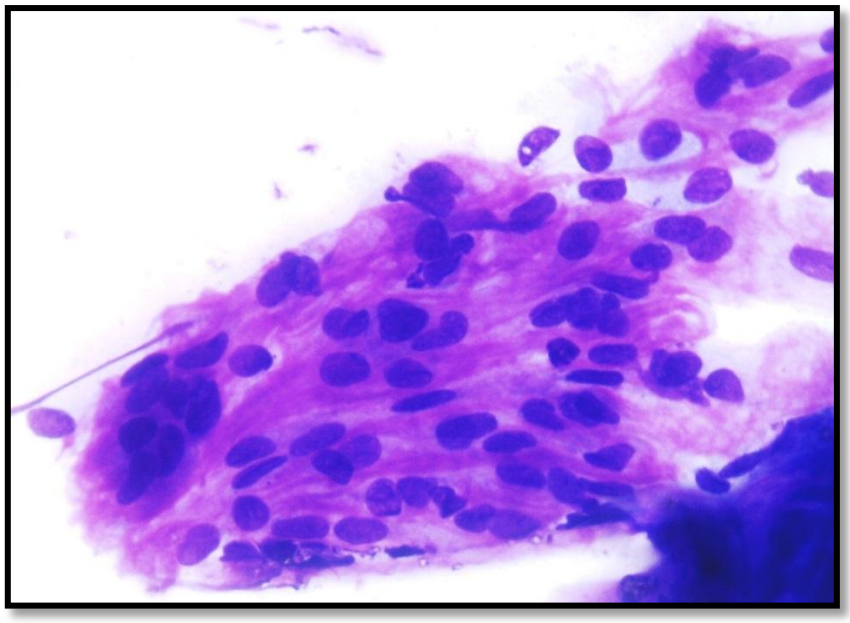

Figure 21: Metaplastic Cytoplasm in a case of Colloid Nodule.

Giemsa stain; 40X

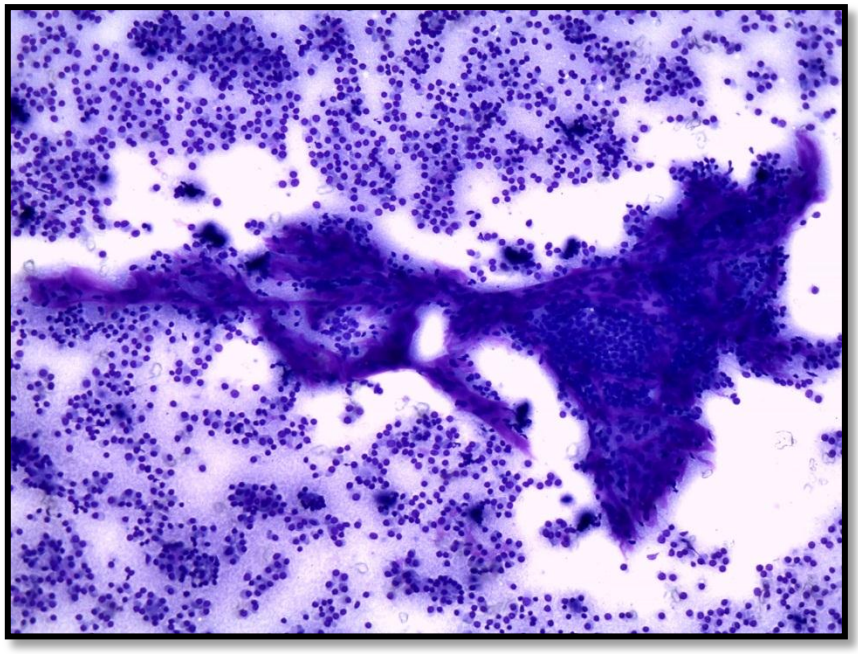

Figure 22: Papillary fragment in Follicular neoplasm. Giemsa stain; 10X

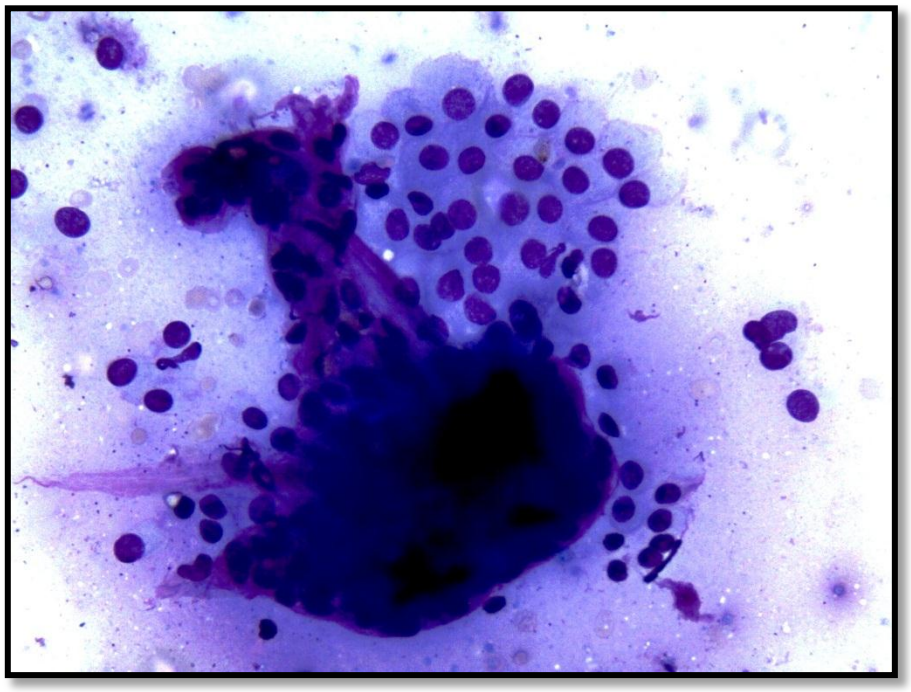

Figure 23: Three dimensional fragment in Hurthle cell neoplasm. Giemsa stain; 40X